BLOOD FLOW IN HUMAN TEETH USING LASER DOPPLER FLOWMETRY; EVALUATION OF REPEATABILITY, ENVIRONMENTAL INFLUENCES AND LEFORT SURGERY

KIMSEY KAY ANDERSON, D.D.S.

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Introduction

As more adult patients seek orthodontic care, orthognathic surgery is included in the treatment plan. As the incidence of surgical procedures increases the interest in the relationship between the surgical procedure and sequelea becomes more defined. The morbidity of any surgical procedure is directly effected by the vascular perfusion of the surgical tissues. When the primary blood supply to the dental pulp is injured or interrupted, collateral circulation does not ensure sufficient perfusion to the pulpal tissues in order to avoid subsequent degenerative pulpal changes (Zisser et al., 1982). Since vitality of the pulp is dependent upon an adequate blood supply, it has been suggested that loss of tooth vitality may be one of the most sensitive indicators of decreased perfusion following maxillary orthognathic surgery (Lanigan et al., 1990).

With the advent of laser Doppler flowmetry (LDF) there are new opportunities for the clinical researcher to obtain perfusion information with a continuous, non-invasive method of monitoring pulpal blood flow (Gazelius et al., 1986; 1988; Olgart et al., 1988; Vongsavan et al., 1993). Until recently, investigating intraoperative pulpal blood flow (PBF) in the orthognathic patient has been difficult due to the limitations of continuous tooth movement, orthodontic apparatus, space limitations, sterile surgical environment and interference with the surgical procedure. Dr. Marianne DiCerbo addressed these limitations and constructed an orthodontic probe holder that allows PBF recordings to be collected preoperatively and

intraoperatively (DiCerbo, 1992). This pilot study was the first its show success with the appliance in a surgical environment.

The credibility of a LDF measurement depends on its repeatability, reproducibility, and accuracy under a given experimental condition. In this case a prerequisite for a successful surgical experiment is our confidence that repeatible, reproducible, and accurate LDF recordings of PBF can be obtained in human teeth. The first portion of this research investigates the repeatability and reproducibility of placement of the LDF probe for human PBF recordings when using the orthodontic probe holder. This study also investigates the individual variation that is associated when this technique is used to collect data.

A second requirement to consistent reliable LDF measurements is the mitigation of everyday environmental factors. The next portion of this research investigates several variables that previous investigators have mentioned in their work that could be associated with variation in LDF recordings.

As the first two phases of this project are evaluated and the PBF of two different maxillary surgical procedures are investigated, the validity of using this technique to obtain the perioperative and intraoperative PBF data can be critiqued. Then it is possible to evaluate the advantages and disadvantages of this protocol.

PULPAL BLOOD FLOW IN HUMANS USING LASER DOPPLER FLOWMETRY : A TECHNIQUE ALLOWING STABILITY AND REPEATABILITY OF PULPAL BLOOD FLOW DURING SURGICAL MANIPULATIONS

Abstract

Purpose: To validate the repeatability of pulpal blood flow (PBF) in human teeth with the laser Doppler flowmetry (LDF) technique using a repeatable method of attachment of the laser Doppler probe. To evaluate the variation of LDF measurements over time periods that range from hours to days

Methods: In a prospective study, the PBF was recorded from a maxillary central incisor (randomly selected) at a site directly gingival to the orthodontic bracket of 3 subjects. Measurements were made 8 times over a two week period and every 30 minutes over 4 hours for 8 recordings.

Results and Conclusions: The present study indicates that there is only a slight difference between the temporal variability of the laser Doppler measures over the course of four hours (intraday variability) or two weeks (interday variability). Furthermore, it appears that subjects do indeed vary in their mean value recordings and may vary widely in their respective signal to noise ratios.

Key word index: laser Doppler flowmetry, orthodontics, pulpal blood flow, dental pulp, human

Introduction

The use of laser Doppler flowmetry (LDF) affords new opportunities for the clinical researcher that have never before been available by providing a continuous, non-invasive method of monitoring pulpal blood flow (Gazelius et al., 1986; 1988; Olgart et al., 1988; Vongsavan et al., 1993). Dental researchers have employed the LDF technique to measure pulpal blood flow (PBF) to assess blood perfusion to the pulp and evaluate the vascularity of the surrounding bone and supporting structures (Zisser et al., 1982; Ramsay et al., 1991, 1991a; DiCerbo, 1992; Vongsavan et al., 1993; Geylikman et al., 1995). A critical requirement to consistent repeatable LDF measurements is the mitigation of everyday environmental factors. This research investigates a technique to ensure repeatability and reproducibility of LDF probe placement for human PBF recordings.

Laser Doppler flowmetry employs a monochromatic laser to measure the flux of blood cells through tissue by measuring the Doppler shift and intensity of reflected laser light as a result of scatter from the moving red blood cells (Figure 1). A low power (2mW) helium-neon (He-Ne) laser emits red light at wavelength 632.8 nm. The light is conducted from the laser towards the area of interest by means of a fiberoptic conductor. The incident light illuminates the surrounding tissue to a depth of approximately 1 mm or greater depending upon the translucency of the tissue to red light. When the incident light is scattered by the moving blood cells, the reflected light exhibits a shift in frequency proportional to the velocity of the blood cells flowing through the area of interest. In addition, the intensity of the Doppler-shifted light is proportional to the total number of blood cells in the measurement

region. Laser light that scatters off the surrounding stationary tissue does not exhibit a Doppler shift. Reflected light, both Doppler-shifted and unshifted, is collected by a two fiberoptic sensors on the probe and is combined with unshifted laser light. This "mixing" process produces a low frequency interference effect that can be measured with standard electronic techniques. The flux of blood through the measurement region can be derived from the measurement of the interference effect and intensity of the reflected shifted light. Further information on this technique can be found in Öberg et al., (1984). Critical to this technique is that the structures and tissues have adequate translucency to allow the passage of the laser to the underlying vasculature. Unfortunately, due to variability in tissue consistency, thickness, and translucency between individuals that are enrolled in any given study, the laser Doppler measures between individuals cannot be directly compared. This variability among individuals lends the use of laser Doppler to longitudinal rather than cross-sectional study design. Therefore, when comparing the data, each subject serves as their own control and the difference in PBF is shown as the percent of change from the subject's normalized value.

Identified sources of variability in the LDF measurements include stability, (Vongsavan et al., 1993; Ingolfsson et al., 1993) repeatable and accurate placement of the probe and temporal variation (Gazelius et al., 1986, Ramsay et al., 1991; Ingolfsson et al., 1993). Gazelius et al., (1986) studied the effects of repeatable probe placement using a modified rubber dam clamp for probe placement in their study. They reported an individual coefficient of variation range of 2% to 14% which they postulated was due to error in probe replacement and external influences such as temperature. Ramsay et al.,

(1991) conducted a study to investigate the repeatability of the laser Doppler recordings over time. They utilized a removable plastic splint with precisely drilled holes to support the probe and provide repeatability for probe placement during laser Doppler recordings. Their results suggested that reliable longitudinal data requires accurate repositioning of the probe and control of environmental conditions to minimize temporal variation. Additionally, Ingolfsson et al., (1993) employed a rubber base splint to hold the probe for stable positioning during recordings. Their results reveal a 7.9% to 10.3% coefficient of variation which they felt was due to the differences in probe placement on the tooth surface. DiCerbo, (1992) reports a difference of 10 - 16% over a 4-7 day period of two repeated laser Doppler measurements. Her probe placement technique provides greater control over placement and repeatability and has been adopted for this study with minor modifications.

Orthodontic patients undergo three dimensional changes in tooth movement over time, and in order to conduct longitudinal studies on them, an accurate technique to ensure repeatable and accurate probe placement for these subjects is required. Additional requirements of the probe holder are that it be stable during placement, measurement and removal and it be able to be sterilized by heat as this would enable use during surgery. If used during surgery it should also be small enough to remain attached to the subject's archwire between measurement sessions and still not interfere with the surgeon's ability to perform any procedure. Repeatability and placement of the probe holder are the two primary research objectives of this study. The first objective is to validate the repeatability of the LDF technique to measure PBF using this repeatable method of stabilization and attachment of the laser Doppler probe. The second objective is to evaluate the variation of LDF

measurements over time periods that range from hours to days using this placement technique.

Materials and Methods

Subjects - The volunteers for this study were dental students from the School of Dental Medicine at the University of Pennsylvania. The study protocol and informed consent procedure were approved by the Human Resource Committee of the University of Pennsylvania. The three subjects ranged in age from 24 to 30 years. Randomized (right or left) selection of nonrestored central incisor teeth were used for the PBF recordings.

Apparatus - A laser Doppler flowmeter (Periflux PF-3®, Perimed, Stockholm, Sweden) was employed to measure the PBF. A fiberoptic probe designed to measure PBF on teeth (Perimed model 316, Perimed) was held in a rigid position against the tooth using an orthodontic probe holder developed at Columbia University School of Dental and Oral Surgery by DiCerbo, (1992). The orthodontic probe holder was fabricated using two soldered hexagonal archwire locks (Figure 2) (RM-locks®, Rocky Mountain Orthodontics (RMO), Denver, Colorado, USA) and a 1.0 inch piece of rectangular stainless steel orthodontic wire (.021 x .025 inch). One end of rectangular orthodontic wire was adapted to fit closely around the end of the probe (1.9 mm). The rectangular wire was attached to the soldered locks via a hex wrench (RMO). The probe holder in turn was attached to the subject's archwire so that it was centered mesiodistally on the labial surface of the selected incisor tooth and directly gingival to the orthodontic bracket. Excess wire on the opposite side was bent into a small loop to ensure retention and stabilization. A stainless steel ligature was passed through the loop and

ligated around the subject's archwire and bracket to further ensure minimal movement of the probe during any procedure (Figure 3). Each of these subjects had two straightwire orthodontic brackets passively bonded to their maxillary central incisors for two weeks. An orthodontic-probe holder was fabricated for each selected maxillary central incisor for each subject. Adjustments of the orthodontic-probe holder were made to assure that the laser Doppler probe was placed in the desired location and at a right angle to the surface of the tooth (Figure 4-6). Once the probe holder was positioned, the laser Doppler probe was inserted into the orthodontic probe holder and positioned within 0.5 millimeter of the tooth. The distances between the laser Doppler probe and the mesial and incisal edges of the tooth were recorded with a millimeter ruler and documented. The probe was visually inspected to ensure that it was at a right angle to the surface of the tooth and that all plaque and residual bonding material was removed. After each set of measurements, the probe holder was removed and was reapplied at subsequent sessions. The orthodontic probe holders, hex wrench and any necessary orthodontic pliers were cold or dry heat sterilized prior to each measurement session in accordance with clinic protocol. The laser Doppler probe was sterilized according to manufacturer's specifications.

At the beginning of each measurement session, the laser Doppler system was calibrated using a zero motility standard (Delrin ring®, Perimed, Stockholm, Sweden) in conjunction with the manufacturer's recommended calibration technique. Periodically, during the two week measurement period, the laser Doppler system was evaluated for stability and recalibrated to a motility standard (Periflux PF100®, Perimed). The laser Doppler flowmeter time constant was set at 0.2 seconds, the artifact filter switch was activated and

PBF data was collected on the narrow band setting. Additionally, during data recording sessions, movement of the probe, fiberoptic cables and subject where minimized to the greatest extent possible.

Procedures - The subjects were placed in a supine position in the dental chair and asked to rest for 5 minutes. The subject's blood pressure and pulse were recorded prior to data collection and all recordings were made at approximately the same time of each day to control for possible circadian rhythm variation. The orthodontic probe holder was positioned on the archwire and the laser probe attached. As suggested by Gazelius et al., (1986), all output signals were recorded on the computer after 3-4 minutes of recording time had elapsed. PBF data was collected for a period of three consecutive minutes then the probe holder and passive wire were removed. A series of eight measurements were taken from each of the three subjects over a two week period (interday). Additionally, between the seventh and eighth recordings, a measurement session lasting four hours was conducted with a PBF measurement being taken every thirty minutes (intraday). All recordings were taken in the same treatment room in the same dental chair, temperature, and under similar lighting conditions (no natural light was allowed in the room during recordings). The position of the dental chair was preset for reproducibility. Additionally, during data recording sessions, movement of the probe, fiberoptic cables and subject where minimized to the greatest possible extent. All data was collected by a single examiner.

Results

Pulpal blood flow data measured by the PF-3 laser Doppler flowmeter were recorded at a rate of 32 signals per second by a laptop computer (Apple

PowerBook 160®, Apple Computer Inc., Cupertino, CA, USA). The laser Doppler flowmeter was connected to the laptop using a standard RS-232 connection. A basic software (LabVIEW®, National Instruments, Austin, TX, USA) together with a software module developed at the University of Washington, Seattle, WA were used to collect, store and analyze the resultant PBF data. The data collected for each of the three minutes was individually averaged and a standard deviation calculated (dropping all artifacts present before calculation). Our aim was to obtain the most accurate measurement at each session, therefore the mean value for the minute with the lowest standard deviation and minimum artifacts recorded is the reported measurement for that three minute session as presented in Table 1. Mean and standard deviation of each series of recordings were calculated. Using these values a percent variation was then derived for each series by dividing the mean by the standard deviation (SD) and multiplying by 100 [(mean/SD) x 100]. This gives an intra individual variability of 13-25%. When comparing standard deviations for each individual for interday and intraday variability we noted that the values were of the same magnitude (i.e. subject A interday 0.48 SD vs. intraday 0.41 SD) as seen on Table 1.

Discussion

The measurements for the previous studies, excluding DiCerbo, were accomplished using removable splints which could elicit force on both the splint and the tooth during placement and removal. This approach can introduce three potential variability sources: lack of repeatability in splint placement, lack of splint material stability, and lack of a consistent contact or distance at the probe/tooth interface. Using the probe placement technique

outlined in this paper, if orthodontic tooth movement occurred or orthodontic appliances were modified (i.e. bracket position) during the course of a study, measurements taken to locate the probe position could have been used to adjust the orthodontic probe holder to ensure reproducible probe placement for minimal effort. Fortunately, this was not necessary during the limited course of this study.

Every attempt was made to standardize the measurement condition and to minimize movement artifacts. The movement artifacts were considered extremely important, as movement of the probe or the subject produced changes in the output measurements resulting in an artifact. In keeping with previous studies Gazalius et al., (1986) and Ramsay et al., (1991), we noted that some time was needed before a stable measure was achieved. Measurements collected in the third minute often yielded measures with less variability than either of the preceding two minutes. The present study indicates that there is only a slight difference between the temporal variability of the laser Doppler measures over the course of four hours (intraday variability) or two weeks (interday variability). Furthermore it appears that subjects do indeed vary in their mean value recordings and may vary widely in their respective signal to noise ratios.

Gazelius et al., (1986) observed intra-individual temporal variability to range from 2 to 14 % in five subjects over a two month period. They attributed the differences to errors in probe replacement and the influence of factors such as temperature change on the blood flow. However there is no mention of the environmental control that accompanies this study, so we don't know if there was control of subject position, ambient light , or force from rubber dam and cervical clamp that could limit normal temporal

variability. In the present study probe position was strictly standardized according to the methods reported previously. In addition background variables such as pulse rate, blood pressure, ambient light, and temperature did not differ greatly among sessions. Accordingly, our data suggests a more liberal range for intra-individual variability of between 13 to 25% when utilizing a probe holder as we have described. Also our data revealed only a slight difference in variability within a subject when intraday and interday variability were compared. This information is particularly useful when an investigation using stimulus-response or longitudinal measures is carried out.

The results of our study suggest that the laser Doppler technique has an inherent variability associated with temporal measures. What appears most intriguing is that the variability appears to have the same magnitude independent of the length of temporal interval (four hours versus two weeks). Variability can come from the surrounding environment, probe positioning, and actual changes in the PBF. The literature has not provided significant information concerning the actual nature of PBF when viewing it with holistic indicators such as laser Doppler. This may suggest that there are mechanisms that operate to vary overall pulpal perfusion that are not currently accounted for.

In summery, the advantages of this probe holder technique are; First, it can be heat sterilized. Second, it is fully adjustable and can be modified if tooth movement occurs or orthodontic appliances are modified between measurement sessions. Third, the holder and its contingent parts are of stable materials. Forth, it is stable during placement, measurements, and upon removal. Fifth, it places no force on the tooth or surrounding tissues during

placement, measurements, or removal thereby influencing the data collected. Sixth, all parts are accessible visually for verification of position and integrity during data collection procedures. It's disadvantages include the initial fitting and wire adaptation are time consuming at the chairside, and unless the solder joint is fabricated by a vacuum laser soldering technique or similar manner as accomplished by RMO, it could have similar results to the silver solder joint used during the pilot study that proved breakable with excessive force. The locks used in this study were tested to 200 lbs./ in^2. There were no problems during our study with breakage.

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The opinions and conclusions in this paper are those of the authors and are not intended to represent the official position of the Department of Defense, United States Air Force, or any other government agency.

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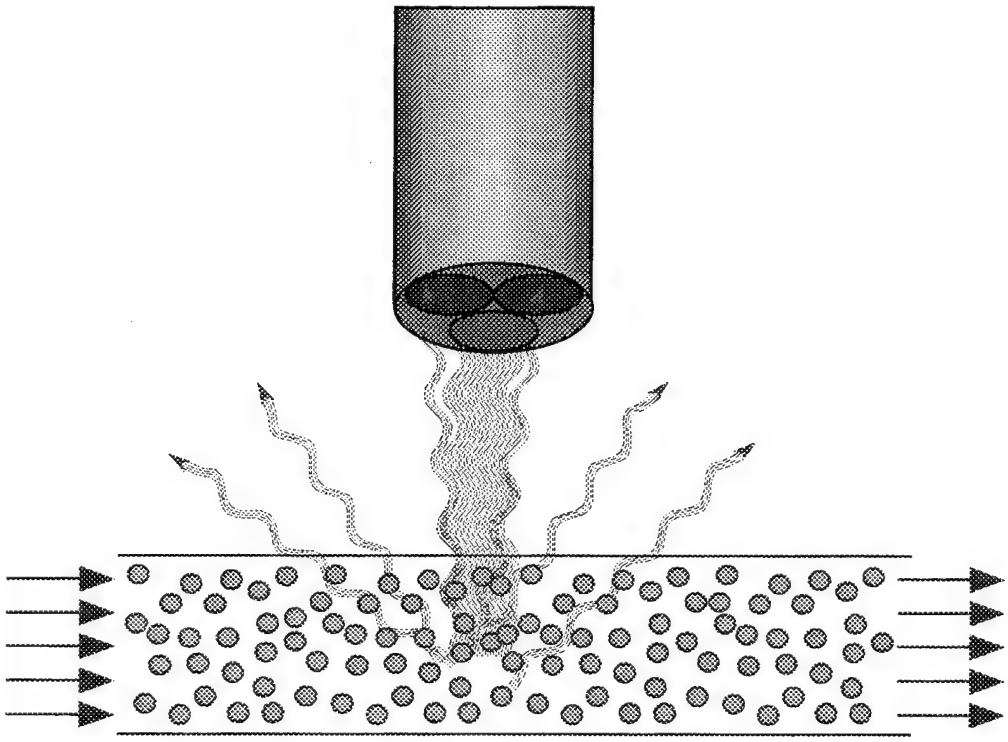

Figure 1: Schematic of Laser Doppler Flowmetry

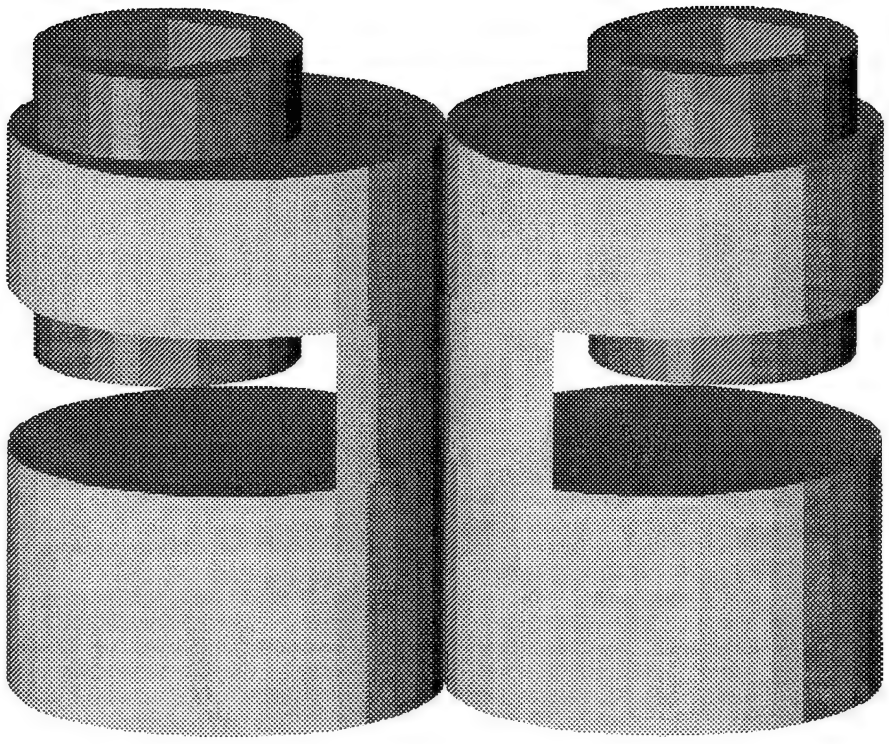

Figure 2: Soldered RMO Locks used in the Orthodontic Probe Holder

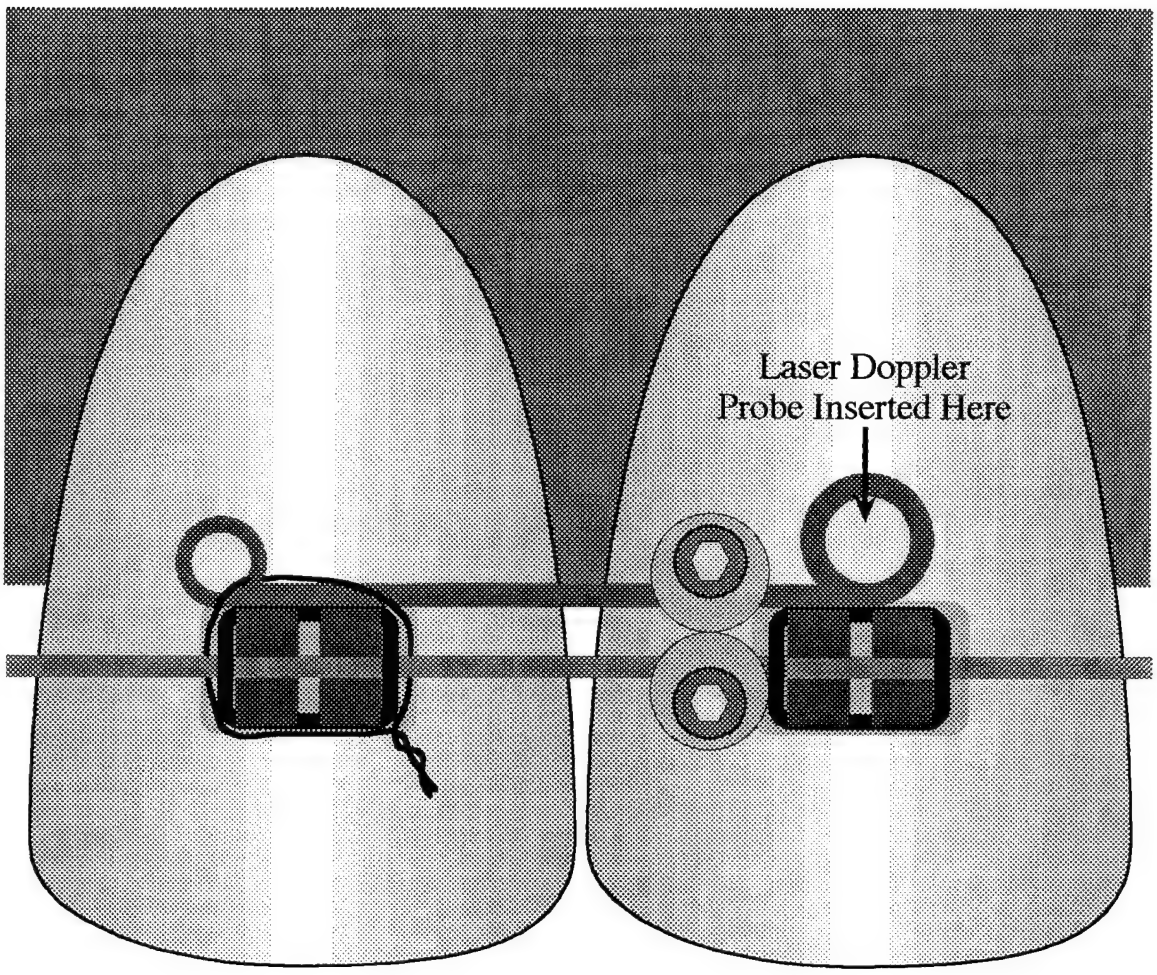

Figure 3: Probe and Probe Holder

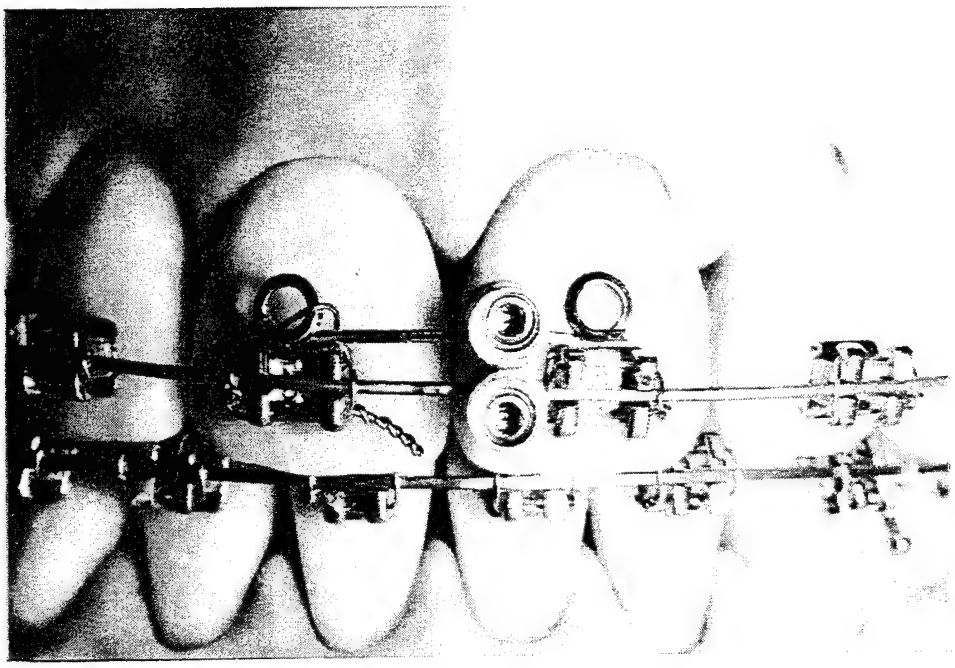

Figure 4: Orthodontic Probe Holder

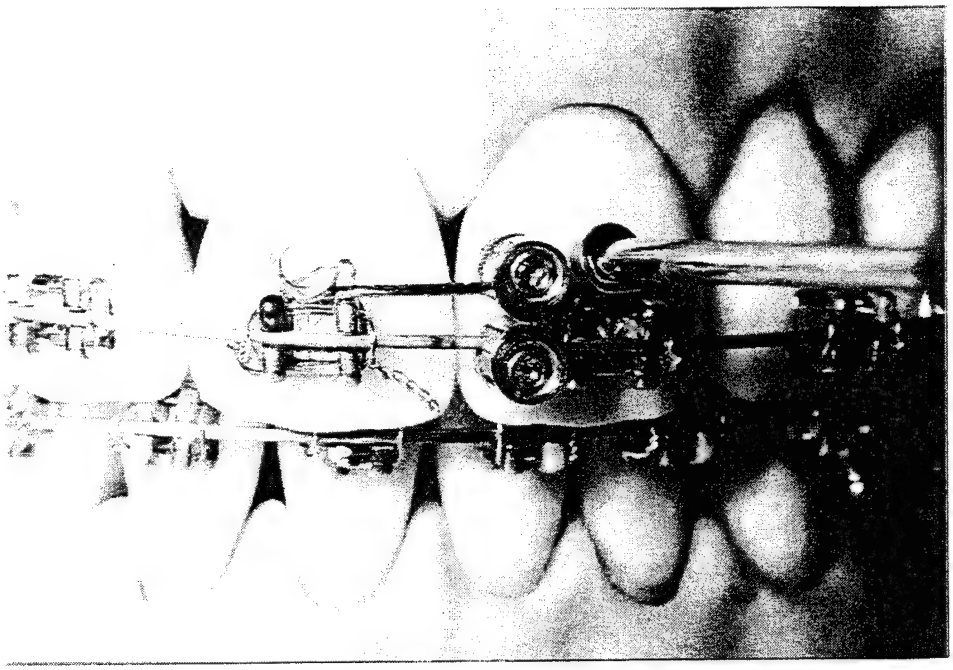

Figure 5: LDF Probe in Probe Holder

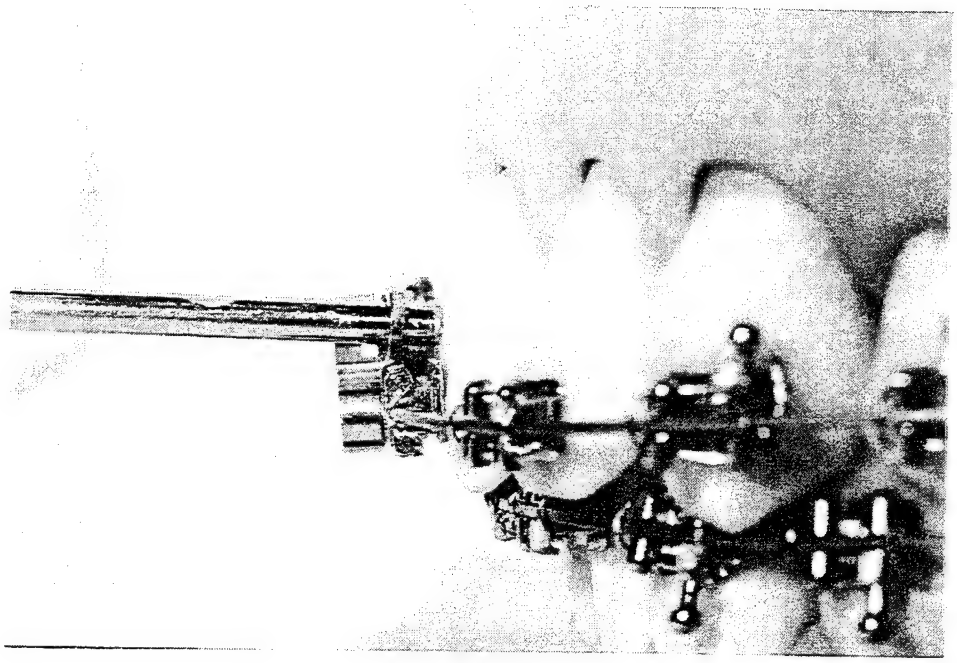

Figure 6: Profile View of LDF Probe in Probe Holder

INTERDAY Recordings

Recordings taken over a two week period

	Subject		
Application	**A**	**B**	**C**
Day 1	2.56	4.24	4.59
Day 2	3.34	5.61	3.97
Day 3	2.83	6.04	3.21
Day 4	3.19	7.41	5.66
Day 5	2.64	6.63	4.82
Day 6	3.91	6.43	3.83
Day 7	2.50	6.41	6.43
Day 8	3.20	6.21	6.40
Mean	3.02	6.12	4.86
Standard Deviation	0.48	0.92	1.20
% Variation	15.91	15.01	24.73

INTRADAY Recordings

Recordings taken over an 8 hour period

	Subject		
Application	**A**	**B**	**C**
Hour 1	2.50	6.21	6.40
Hour 2	2.46	6.30	7.15
Hour 3	3.20	6.41	8.33
Hour 4	2.96	5.91	8.79
Hour 5	3.04	4.63	5.45
Hour 6	3.35	4.97	4.89
Hour 7	3.55	6.97	7.91
Hour 8		5.97	4.50
Mean	3.01	5.92	6.68
Standard Deviation	0.41	0.77	1.62
% Variation	13.63	12.99	24.32

Table 1: Interday and Intraday Pulpal Blood Flow Measurements

PULPAL BLOOD FLOW USING LASER DOPPLER FLOWMETRY: ENVIRONMENTAL EFFECT STUDIES

Abstract

Purpose: To evaluate the effects of four environmental factors on laser Doppler flowmetry recordings of pulpal blood flow(PBF); 1) probe position relative to the gingiva, 2) peripheral laser scatter due to gingival blood flow, 3) overhead dental lighting, and 4) subject position. To also evaluate the effects of laterally generated orthodontic forces on PBF.

Methods: In a prospective study the PBF was recorded on two sites directly gingival to the orthodontic bracket on a randomly selected maxillary central incisor of 27 subjects while testing the environment effects listed above.

Results: When the site of the probe position was moved gingivally, the output signal increased an average 29% on the tooth being evaluated. When foil was placed over the gingiva a 0.84% reduction resulted. When directing the dental light on the probe site the output signal decreased an average of 56% and the output signals recorded with the subject in the supine position as compared to those recorded with the subject in the 90 degree upright seated position was increased 7.5%. When laterally directed orthodontic force was placed a statistically significant 5% decrease in PBF was found.

Conclusion: The results of this study confirmed that moving the probe site location in the gingival direction increases the recording measurement of the PBF of the tooth being evaluated. There was no statistically significant difference in the output signals recorded with foil over the adjacent gingiva. The application of a bright dental light to the site location affects the accuracy of the LDF recording and this effect can be a major factor in the interpretation

of study results if not controlled in the study design. Recordings with the subject in different positions may effect LDF measurements and should be controlled for until further studies are accomplished. Finally, the application of 134 grams of laterally directed force decreases PBF a statistically significant 5% as compared to PBF recorded before the force was applied.

Key word index: laser Doppler flowmetry, orthodontics, pulpal blood flow, dental pulp, human

Introduction

The use of laser Doppler flowmetry (LDF) affords new opportunities for the clinical researcher that have never before been available by providing a continuous, non-invasive method of monitoring pulpal blood flow (Gazelius et al., 1986; 1988; Olgart et al., 1988; Vongsavan et al., 1993). Dental researchers have employed the LDF technique to measure pulpal blood flow (PBF) and assess blood perfusion to the pulp and evaluate the vascularity of the surrounding bone and supporting structures (Ramsay et al., 1991, 1991a; Vongsavan et al., 1993; Zisser et al., 1982; DiCerbo, 1992). A critical requirement to consistent repeatable LDF measurements is the mitigation of everyday environmental factors. This research evaluates a number of environmental factors which can affect the validity and repeatability of LDF measurements and proposes straightforward techniques which can be employed to mitigate the environmental influences.

Laser Doppler flowmetry employs a monochromatic laser to measure the flux of blood cells through tissue by measuring the Doppler shift and intensity of reflected laser light as a result of scatter from the moving red blood cells (Figure 1). A low power (2mW) helium-neon (He-Ne) laser emits red light at wavelength 632.8 nm. The light is conducted from the laser toward the area of interest by means of a fiberoptic conductor. The incident light illuminates the surrounding tissue to a depth of approximately 1 mm or greater depending upon the translucency of the tissue to red light. When the incident light is scattered by the moving blood cells, the reflected light exhibits a shift in frequency proportional to the velocity of the blood cells flowing through the area of interest. In addition, the intensity of the Doppler-shifted light is proportional to the total number of blood cells in the measurement

region. Laser light that scatters off the surrounding stationary tissue does not exhibit a Doppler shift. Reflected light, both Doppler-shifted and unshifted, is collected by a two fiberoptic sensors on the probe and is combined with unshifted laser light. This "mixing" process produces a low frequency interference effect that can be measured with standard electronic techniques. The flux of blood through the measurement region can be derived from the measurement of the interference effect and intensity of the reflected shifted light. Further information on this technique can be found in Öberg et al., (1984). Critical to this technique is that the structures and tissues have adequate translucency to allow the passage of the laser to the underlying vasculature. Unfortunately, due to variability in tissue consistency, thickness, and translucency between individuals that are enrolled in any given study, the laser Doppler measures between individuals cannot be directly compared. This variability among individuals lends the use of laser Doppler to longitudinal rather than cross-sectional study design. Therefore, when comparing the data, each subject serves as their own control and the difference in PBF is shown as the percent of change from the subject's normalized value.

In addition to subject tissue variability, repeatable probe placement and temporal variation, other sources of measurement variability associated with LDF technique have been identified and studied. These include contribution due to gingival blood flow, enamel-dentin thickness (Ingolfsson et al., 1993), subject position (DiCerbo, 1992), probe position (Ramsay et al., 1991), stability and high intensity light (Vongsavan et al., 1993). Gazelius et al., (1986) employed a modified rubber dam clamp for probe placement in their study. Additionally, they used green rubber dam material to minimize scattering of

peripheral laser light from surrounding tissues. An individual coefficient of variation ranging from 2% to 14% was reported. It was postulated this variability was due to errors in replacement of the probe and external influences such as temperature. When Ramsay et al., (1991) conducted a study to investigate the repeatability of the laser Doppler measurements a removable plastic splint with precisely drilled holes was used to support the probe. This appliance provided repeatability for placement of the probe in several positions during the laser Doppler measurement. Their results suggested that reliable longitudinal data requires accurate repositioning of the probe and control of environmental conditions to minimize temporal variation. Finally, Ingolfsson et al., (1993) employed a rubber base splint to hold the probe for stable positioning during recordings. Their results reveal a 7.9% to 10.3% coefficient of variation which they attributed to differences in probe placement on the tooth surface. This and previous research indicates that everyday environmental factors can have a significant impact on the repeatability and variability of LDF measurements.

The purpose of this research is two fold: First, evaluate the effects of four environmental factors on laser Doppler flowmetry recordings: probe position relative to the gingiva, peripheral laser scatter due to gingival blood flow, overhead dental lighting, and subject position. Second, evaluate the effects of laterally generated orthodontic forces on PBF.

Materials and Methods

Subjects - Volunteers for this study were all orthodontic subjects currently undergoing treatment for routine orthodontics at the Orthodontic Department at the University of Pennsylvania School of Dental Medicine.

The study protocol and informed consent procedure were approved by the Human Resource Committee of the University of Pennsylvania. A total of 27 subjects, all treated by one resident, were included in this study. Their ages ranged from 13 to 53 years of age, with a mean age of 21.2 years and standard deviation of 10.5 years. All were undergoing orthodontic treatment which required resin bonded bracket placement on the maxillary incisors. Randomized (right or left) selection of nonrestored central incisor teeth were used for the PBF recordings.

Apparatus - A laser Doppler flowmeter (Periflux PF-3®, Perimed, Stockholm, Sweden) was employed to measure the PBF. A fiberoptic probe designed to measure PBF on teeth (Perimed® model 316, Perimed) was held in a rigid position against the tooth using an orthodontic probe holder developed at Columbia University School of Dental and Oral Surgery by DiCerbo, (1992). The orthodontic probe holder was fabricated using two soldered hexagonal archwire locks (Figure 2) (RM-locks®, Rocky Mountain Orthodontics (RMO), Denver, Colorado, USA) and a 1.0 inch piece of rectangular stainless steel orthodontic wire (.021 x .025 inch). One end of rectangular orthodontic wire was adapted to fit closely around the end of the probe (1.9 mm). The rectangular wire was attached to the soldered locks via a hex wrench (RMO). The probe holder in turn was attached to the subject's archwire so that it was centered mesiodistally on the labial surface of the tooth of interest and directly gingival to the orthodontic bracket. Excess wire on the opposite side was bent into a small loop to ensure retention. A stainless steel ligature was passed through the loop and ligated around the subject's archwire and bracket to further ensure minimal movement of the probe during any procedure (Figure 3). The distances between the laser

Doppler probe and the mesial and incisal edges of the tooth were recorded with a millimeter ruler and documented. The probe was visually inspected to ensure that it was positioned at a right angle to the tooth surface and that all plaque and residual bonding material was removed An orthodontic-probe holder was fabricated for the selected maxillary central incisor for each subject. After each set of measurements, the probe holder was removed and reapplied at subsequent sessions as stated in Paper I. The orthodontic probe holders, hex wrench and any necessary orthodontic pliers were cold or dry heat sterilized prior to each measurement session in accordance with clinic protocol. The laser Doppler probe was sterilized according to manufacturer's specifications.

At the beginning of each measurement session, the laser Doppler system was calibrated using a zero motility standard (Delrin ring®, Perimed, Stockholm, Sweden) in conjunction with the manufacturer's recommended calibration technique. Periodically, during the six month measurement period, the laser Doppler system was evaluated for stability and recalibrated to a motility standard (Periflux® PF100, Perimed). The laser Doppler flowmeter time constant was set at 0.2 seconds, the artifact filter switch was activated and pulpal blood flow data was collected on the narrow band setting. Additionally, during data recording sessions, movement of the probe, fiberoptic cables and subject where minimized to the greatest extent possible.

Procedures - All measurements were taken at the beginning of a routine orthodontic appointment, which was at least 4 weeks from the last orthodontic adjustment. The average time between measurement sessions was 36 days. The nature of the orthodontic treatment was limited and not expected to influence pulpal perfusion in the maxillary central incisor area.

Three to five sets of data were obtained on each subject. All recordings were taken in the same treatment room in the same dental chair and to the greatest extent possible under similar lighting conditions (no natural light was allowed in the room during recordings) and temperature. The position of the dental chair was preset for reproducibility. The subjects were placed in a supine position in the dental chair and asked to rest for 5 minutes. At the end of this period, the subject's blood pressure and pulse were recorded. The appropriate orthodontic probe holder was positioned on the archwire and the laser probe attached. As suggested by Gazelius et al., (1986), all output signals were recorded on the computer after 3-4 minutes of recording time had elapsed. All data was collected by a single examiner.

Four sets of data were collected during each session and were used to evaluate the effects of the four environmental factors and lateral orthodontic forces on PBF. Both control and environmental (factor) recordings were collected for 60 second periods and then an average perfusion recording was obtained along with the associated standard deviation. Recordings were taken in a supine position except for the environmental recording to evaluate the effect of subject position. All environmental factor recordings were taken with the probe at Site 1 except the environmental recording for probe position which was taken at Site 2 (Figure 3). Each subject had 2 separate orthodontic probe holders fabricated, separately adjusted for Site 1 and 2 measurement locations. The following series of measurements were conducted during each measurement session:

Gingival Blood Flow : This measurement series was conducted to evaluate the affect of laser light scattered from the gingiva during the PBF

recordings. All recordings were taken at Site 2 and were conducted in two stages:

Stage 1: Control: A recording conducted to measure the PBF at Site 2.

Stage 2: Factor: Foil was contoured and placed over the adjacent gingiva to ensure no laser light was scattered from the gingiva back to the laser probe.

Dental Light : This measurement series was conducted to evaluate the effect of a dental unit light shining on the probe measurement area. All recordings were taken at Site 1 and was conducted in two stages:

Stage 1: Control: The dental unit light was off and a PBF measurement was taken.

Stage 2: Factor: An ADEC Cascade 2000 dental unit light was turned on at a distance of 18 inches from the probe site, perpendicular to the subject's tooth and used to illuminate the probe measurement area.

Subject Position : This measurement series was conducted to evaluate the effect of subject position during the measurement period. The control recording used for the dental light study was also used for this measurement series. This recording was conducted with the subject in a supine position and conducted in one stage:

Stage 1: Factor: A PBF recording was conducted with the subject in a 90 degree upright sitting position.

Subsequent to the environmental measurements, a measurement series was conducted to evaluate the effect of lateral orthodontic force. Each

set of measurements consisted of a series of three recordings to evaluate the influence of this factor. All orthodontic factor recordings were taken with the probe at Site 1.

Lateral Force : This measurement series was conducted to evaluate the effect of lateral orthodontic force on the central incisor being used for the PBF measurement. This series was conducted in three stages:

Stage 1: Control: A PBF recording was conducted with the subject in a supine position with no lateral force applied to the tooth

Stage 2: Factor: A PBF recording was conducted with the subject in a supine position. A lateral force of 134 grams (1.31 N) was applied to the tooth. The lateral force was produced by a spring placed between the subject's two central incisors. At each appointment the distance between the subject's two central incisor teeth was measured and a preadjusted spring calibrated to deliver 134 grams at that distance was used to deliver the lateral force.

Stage 3: Control: A PBF recording was conducted after the lateral force was removed with the subject in a supine position. This was taken to determine if there was any residual effect due to the lateral pressure.

Results

PBF data measured by the PF-3 LDF was recorded at a rate of 32 signals per second by a laptop computer (Apple PowerBook 160®, Apple Computer Inc., Cupertino, CA, USA). The laser Doppler flowmeter was connected to the laptop using a standard RS-232 connection. A basic software program (LabVIEW®, National Instruments, Austin, TX, USA) together with a

software module developed at the University of Washington, Seattle, WA were used to collect, store and analyze the resultant PBF data. Analysis of the effect, of environmental factors on the PBF measurements, was accomplished by normalizing the one minute average of the environmental recording, that had the lowest standard deviation and few or no artifacts, by the one minute average control recording. This is done by dividing both the control recording and the factor recording by the average control recording value. This normalizes the control recording to one and provides a ratio change of the factor recording to the control. A two tailed t-test for related measures was employed to determined statistical differences between the two sets of measurement. The effects of lateral force were calculated in a similar manner to the environmental effects. A p-value ≤ 0.05 was chosen as statistically significant. The PBF recordings are presented in Table 1.

The averages of all the normalized recordings were calculated and plotted in Figure 4. When the probe position site was moved 1mm gingivally the output signal increased an average 29% on the tooth being tested. The difference of all the recordings when normalized and averaged results in only a 0.84% reduction when the foil was placed over the gingiva. When directing the dental light on the probe site the output signal was an average 56% lower with the light directed to the tooth being evaluated. The output signals recorded with the subject in the supine position as compared to those recorded with the subject in the 90 degree upright seated position increased 7.5% and was not found to be statistically significant. Finally, the normalized average value for the laterally directed orthodontic force was a 5% decrease in PBF, and was found to be statistically significant.

Of the four environmental factors evaluated, light and probe site, showed statistical significance. The data on the influence of gingival tissue on PBF, and subject position showed no statistical difference. Of the orthodontic forces data collected when the force was applied showed statistical significance. The data collected when the force was removed did not show a statistical difference from the immediately preceding normal values. The results of Student's T-test are presented in Table 1.

Discussion

Consistent with Ramsay et al., (1991) we found that moving the probe site location in the gingival direction 1 millimeter gave an average increase of 29% on the tooth being evaluated. This was found to be a statistically significant difference. Ramsay et al., (1991) suggests that the increase in PBF measurements when the site is moved gingivally is due to the progressively increasing volume of pulp tissue as this increases toward the cementoenamel junction. Gazalius et al., (1988) also suggests that a change in pulpal volume may account for a reduction in the PBF output signal as they observed this with an increase in secondary dentin formation.

There was no statistically significant difference in the output signals taken with foil over the adjacent gingiva as compared to those taken without foil over the adjacent gingiva. Although we have found this result by only moving the site 1 millimeter gingival there may be a more significant influence if PBF measurements are recorded closer to the adjacent gingival tissue.

The application of a bright dental light to the site location affects the accuracy of the recordings by "swamping" the laser detector with "background

noise". This results in a rapid decrease in the recorded signal output even though the actual blood flow had not appeared to have changed. If there were some other factor causing this "decrease" in PBF it would be expected that the PBF would gradually decrease instead of a rapidly drop as seen in the LDF recording (Figure 5). This effect can be a major factor in the interpretation of study results if not controlled in the study design.

Although the output signals recorded with the subject in the supine position as compared to those recorded with the subject in the 90 degree upright seated position was not found statistically significant, the t-test results of 0.0667 suggest that this environmental factor should be tested further and that repeatability of subject position should be carefully considered in study design.

In the application of lateral force our findings agree with those of McDonald et al., (1994). As they suggest, this may be due to the force placed on apical vessels when laterally loading a tooth thereby effecting PBF. The normalized average value was a 5% decrease in PBF which was found to be a statistically significant difference in the output signals taken with 134 grams of lateral force as compared to the PBF taken before the force was applied. With the removal of lateral force our observations were at variance with those of McDonald et al., (1994). They found a decrease in the PBF for up to 48 hours and it had returned to normal by 72 hours. We found no statistically significant difference in the output signals taken before the force was applied as compared to those taken after the force was applied. However, in this study there was only 1 minute of force and these subjects had been in orthodontics treatment for some time.

In conclusion, as any LDF technique is sensitive to slight movement and pressure, we have seen that it is also sensitive to direct dental light, repeatable position, force on the tooth and possibly the postural position of the subject. In future studies it would be advised that these factors be considered and controlled in the study design for human subjects.

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We thank Dr. Doug Ramsay and Chris Prall for developing the data acquisition and analysis software used for this study and Dr. Mark Franz, PhD for his timeless effort coordinating the software, hardware, and figures used. We are also grateful to Dr. Robert Vanarsdall Jr. the chairman, Department of Orthodontics School of Dental Medicine for continued support and to Saul Katz, PhD for his suggestion to pursue this avenue of investigation. A special thanks to Dr. Karen Coffman and Dr. Lisa Slade-Towns for their help running the computer during data acquisition.

The opinions and conclusions in this paper are those of the authors and are not intended to represent the official position of the Department of Defense, United States Air Force, or any other government agency.

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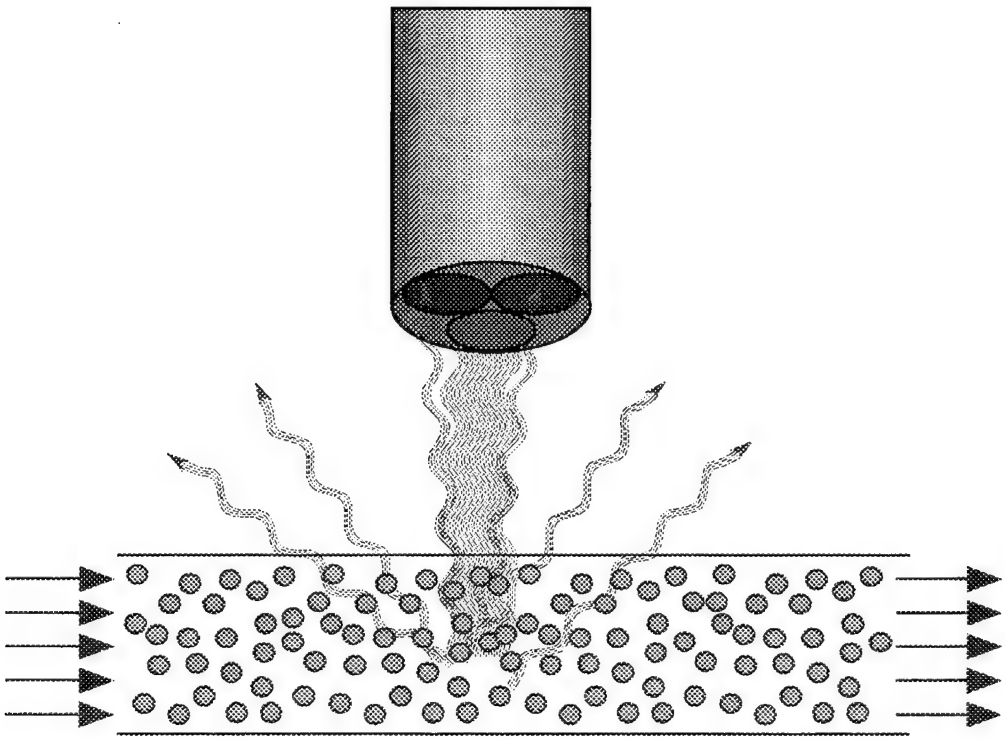

Figure 1: Schematic of Laser Doppler Flowmetry

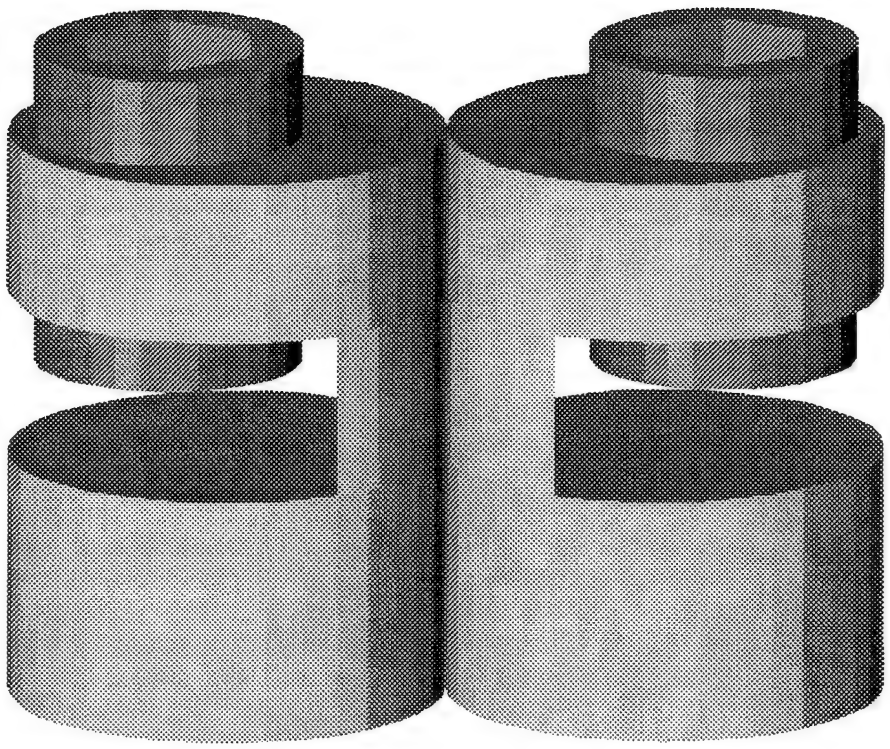

Figure 2: Soldered RMO Locks used in the Orthodontic Probe Holder

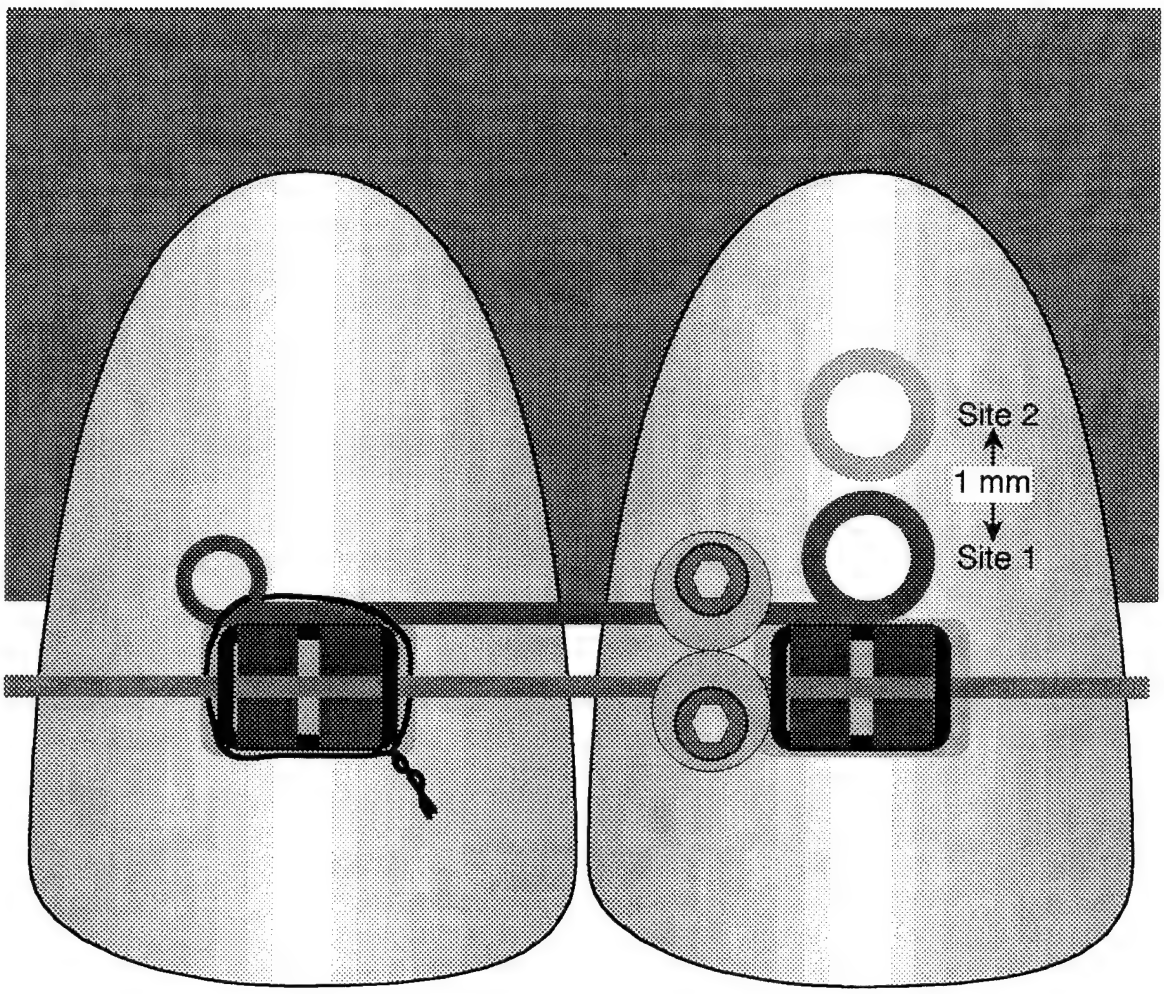

Figure 3: Probe and Measurement Locations

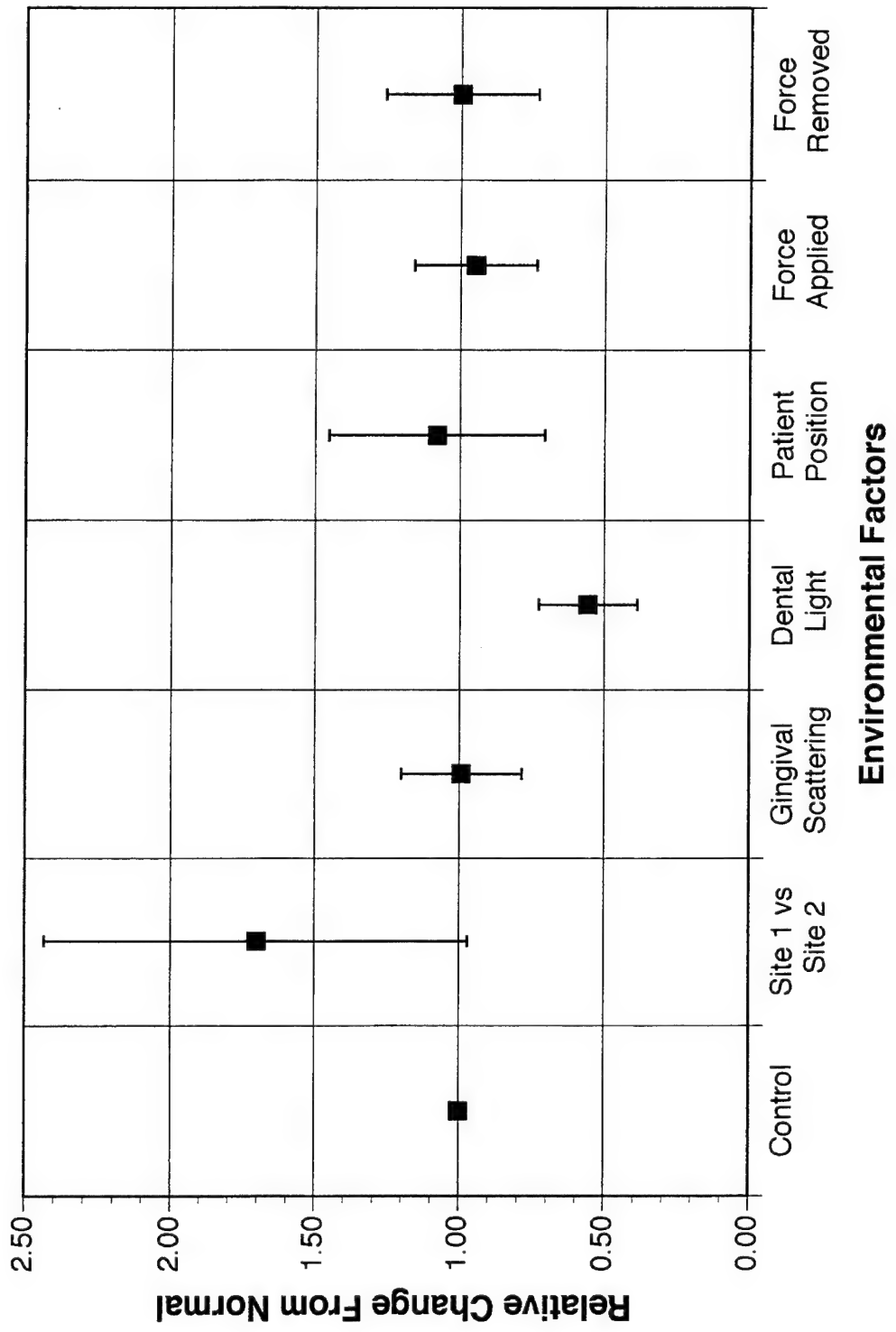

Figure 4: Relative Changes due to Environmental Factors

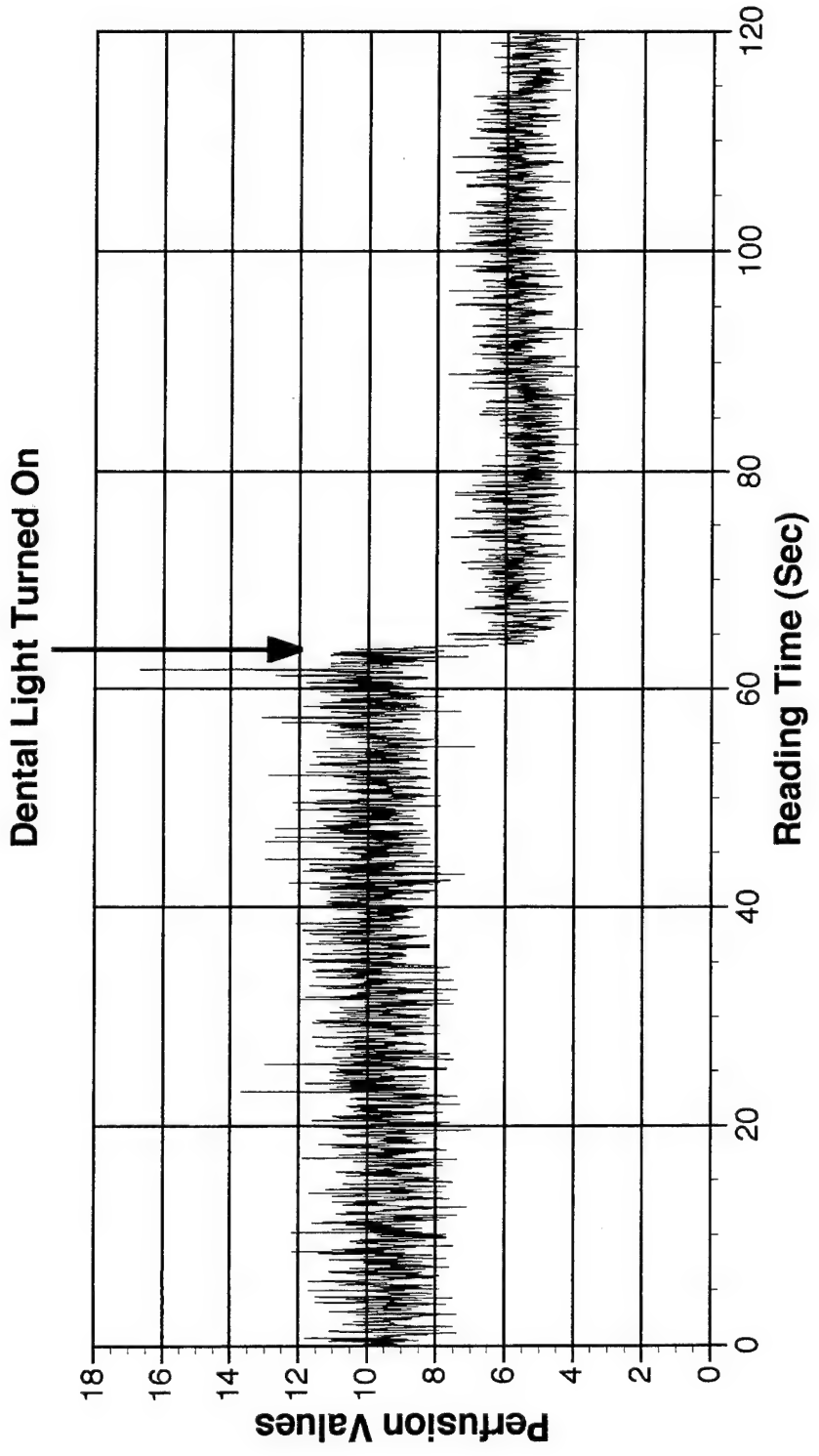

Figure 5: LDF Recordings of subject during addition of dental light

Environmental Factor	p value
2mm vs 1mm	0.000 *
Foil on gingiva	0.760
Dental Light	0.000 *
Lateral Force (add)	0.037 *
Lateral Force(remove)	0.915
Subject Position	0.067

*$p \leq 0.05$ is considered significant.

Table 1: Results of p-value for Student's T-tests for different environmental factors

PULPAL BLOOD FLOW USING LASER DOPPLER FLOWMETRY: AN EVALUATION OF INTRAOPERATIVE AND PERIOPERATIVE CENTRAL INCISOR PERFUSION ASSOCIATED WITH TWO DIFFERENT MAXILLARY OSTEOTOMIES

Abstract

Purpose: To evaluate and compare maxillary central incisor pulpal blood flow (PBF) intra- and perioperatively for both single piece LeFort I osteotomy and surgically assisted rapid maxillary expansion (SRME).

Methods: During this prospective investigation, the PBF was recorded directly gingival to the orthodontic bracket on a randomly selected maxillary central incisor for 6 subjects receiving LeFort I osteotomy and 5 subjects receiving SRME. Measurements were made prior to surgery, 5-7 times during surgery, and 24 hours, 1 week, 2 weeks, 4 weeks, and 4-8 weeks postoperatively.

Results: All PBF values were normalized to the initial operating room recording. In all but one of the subjects the lowest recorded PBF was three minutes after delivery of local anesthesia when the normalized PBF decreased to 40% and 60% in the LeFort I and the SRME groups respectively. The LeFort group showed a small increase of 6% following the completion of the osteotomies and a total of 38% decrease in PBF from the presurgical level just after maxillary downfracture. During subsequent time intervals, the PBF in the LeFort group slowly increased, but not back to the presurgical level until 2-3 months post surgically. In contrast, the SRME group returned to presurgical PBF levels before the surgical procedure was complete and demonstrated hyperemic levels during the intraoperative and postoperative expansion phases. Overall it took significantly longer for the PBF of the LeFort group to return to the presurgical level when compared to the SRME group.

Conclusion: The PBF just after maxillary downfracture in the LeFort group is decreased relative to the initial surgical recording but increased relative to the recording taken after all osteotomies were made. This may be due to surgical stimulation of the downfracture and the subsequent local release of vasoactive nueropeptides resulting in a vasodilatory effect in the dental pulp. Our results suggest that there is a delayed response in the return of PBF to a presurgical level in subjects in the LeFort group as compared to subjects in the SRME group. Due to the length of surgical procedure, this lower PBF in the LeFort group is not likely an effect of the local anesthetic. Our data suggests that sectioning of all of the maxillary articulations following downfracture, prolong the depressed PBF for an extended time. This is first evident during the surgical procedure and continues through the first three months of post surgical healing. In the SRME group the 10% increase in PBF after maximum activation of Haas expansion appliance may be due to the surgical stimulation and the release of vasoactive inflammatory mediators. In addition, postoperatively while expansion is occurring in the SRME group there are also periods of extreme hyperemia that are not seen with the LeFort group. There may be a relationship between hyperemia and expansion, further studies in the areas of nonsurgical expansion (orthopedic RME) and PBF and follow-up comparisons of these same groups may reveal interesting healing effects.

Key word index: laser Doppler flowmetry, orthodontics, pulpal blood flow, dental pulp, human, orthognathic surgery, avascular necrosis.

Introduction

Complications due to insufficient perfusion following maxillary orthognathic surgery have been reported in the literature. Postoperative sequelae due to inadequate perfusion vary greatly in severity and include infection, tooth devitalization, periodontal defects, loss of teeth, soft and hard tissue necrosis, bony nonunion, and/or the loss of entire dentoalveolar segments (Parnes et al., 1972; Sher 1984; Westwood et al., 1975; and Lanigan et al., 1990). Ellingsen et al., (1993) has shown that up to 43% of subjects treated with LeFort I osteotomy exhibit radiographic signs of pulp canal obliteration in one or more teeth with 21% of these subjects also exhibiting pulpal necrosis, as compared to 6% and 10% respectively in non-operated controls. When the primary blood supply to the dental pulp is injured or interrupted, collateral circulation does not ensure sufficient perfusion to the pulpal tissues in order to avoid subsequent degenerative pulpal changes (Zisser et al., 1982). Since vitality of the pulp is dependent upon an adequate blood supply, it has been suggested that loss of tooth vitality may be one of the most sensitive indicators of decreased perfusion following maxillary orthognathic surgery (Lanigan et al., 1990).

Quantitative blood flow studies using microsphere techniques in animals (micro-angiography, isotope fractionation, particle distribution, and H_2 washout) have documented dramatic decreases in pulpal blood flow shortly after maxillary surgery (Meyer et al., 1976 and Indresano et al., 1983). These previous studies, however, have several limitations including: 1) the failure to simulate clinically analogous procedures; (i.e. osteotomies were performed but the osseous segments were not mobilized) 2) inherent limitations in methodology used to study vascular changes (i.e. no standard

microsphere size used in all studies and the impossibility of obtaining longitudinal data on animals that must be sacrificed to obtain information).

The development of laser Doppler flowmetry (LDF) affords new clinical research opportunities by providing a non-invasive and continuous method for monitoring pulpal blood flow (PBF) pre, intra, and postoperatively. LDF employs a monochromatic laser to measure the flux of blood cells through tissue by measuring the Doppler shift and intensity of reflected laser light as a result of scatter from the moving red blood cells (Figure 1). A low power (2mW) helium-neon (He-Ne) laser emits red light at wavelength 632.8 nm. The light is conducted from the laser towards the area of interest by means of a fiberoptic conductor. The incident light illuminates the surrounding tissue to about a depth of 1 mm (or greater depending upon the translucency of the tissue). When the incident light is scattered by the moving blood cells, the reflected light exhibits a shift in frequency proportional to the velocity of the blood cells flowing through the area of interest. In addition, the intensity of the Doppler-shifted light is proportional to the total number of blood cells in the measurement region. Laser light that scatters off the surrounding stationary tissue structure does not exhibit a Doppler shift. Reflected light, both Doppler-shifted and unshifted, is collected by a two fiberoptic sensors on the probe and is combined with unshifted laser light. This "mixing" process produces a low frequency interference effect that can be measured with standard electronic techniques. The flux of blood through the measurement region can be derived from the measurement of the interference effect and intensity of the reflected shifted light. Further information on the specifics of this technique can be found in Öberg et al., (1984). It is critical to this technique that the structures and tissues have

adequate translucency to allow the passage of the laser to the underlying vasculature.

Previous human studies have evaluated the perfusion of the maxilla using either PBF or gingival blood flow (GBF) and both in some studies. When recording PBF the LDF probe is located on the tooth and records the perfusion of the pulp, when recording GBF the LDF probe located on the gingiva recording the perfusion of the gingiva. Other studies evaluating PBF did so pre and postoperatively using vinyl polysiloxane or acrylic splints with holes placed in them to position the Laser Doppler probe (Ramsay et al., 1991a and Geylikman et al., 1995). Dodson et al., (1994) used a von Frey's hair to hold the probe while taking GBF recordings during surgery. The only study using PBF intrasurgically was DiCerbo (1992) in a pilot study when she developed and used the sterilizable and stable orthodontic probe holder technique.

Ramsay et al., (1991a) used LDF to evaluate postoperative changes in PBF following LeFort I osteotomy. Their data showed transient periods of ischemia shortly after surgery and hyperemia during later time intervals. DiCerbo (1992), also using LDF, performed a pilot study on six subjects and measured the intraoperative variations in blood flow during orthognathic surgery. Her results showed significant increases and decreases in PBF in both maxillary and mandibular teeth during and after LeFort I osteotomy and a significant consistent decrease during downward and forward digital pressure of the mobilized maxilla after downfracture. More recently, Geylikman, et. al., (to be published) studied both gingival blood flow (GBF) and PBF before and after LeFort osteotomy. Though they report a statistically significant decrease in GBF during the LeFort I procedure, they did not find the

postsurgical PBF to change significantly. Dodson et al., (1994) showed that maxillary (GBF) decreases significantly over time during LeFort I osteotomy procedure. Results of the above studies identified significant decreases in blood flow of subjects undergoing a single piece LeFort I surgical procedure. To date, there have been no LDF measurements of the PBF during surgically assisted rapid maxillary expansion (SRME).

The overall objective of this research is to build upon the LDF technique developed by DiCerbo and modified by the primary author in two previous studies (Paper I) and effects of environmental factors that may influence recordings (Paper II). Then to employ this LDF technique to measure and compare the intraoperative and perioperative central incisor PBF of subjects undergoing a single piece LeFort I osteotomy (Bell (1973) and Bell et al., (1975)) and surgically assisted rapid maxillary expansion (Betts et al., (submitted for publication)).

The specific aims are 1) to evaluate and compare pulpal blood flow to the central incisor during single piece LeFort I and SRME orthognathic surgical procedures and during a 3 month postoperative healing period, 2) to evaluate pulpal blood flow to the central incisor during intraoperative expansion of the maxilla during SRME, 3) and to correlate post-operative vascular related sequelae with PBF measurements obtained during surgery.

Materials and Methods

Subjects - This prospective study utilized volunteer orthodontic patients who required either a SRME or a LeFort I osteotomy, with or without mandibular osteotomy, to correct jaw deformities. All surgical procedures were performed in a standardized fashion by one of two surgeons (R. F. or

N.B.) from October 1, 1993 to September 30, 1994. The study protocol and informed consent were approved by the Human Resource Committee. Two weeks prior to surgery, the study protocol was explained to candidate subjects. Those that agreed to participate in the study were provided an informed consent for review. All subjects were undergoing orthodontic treatment which required resin bonded bracket placement on the maxillary incisors and those subject undergoing a SRME had placement of a Haas expansion appliance with full palatal coverage. All incisors monitored in this study were either minimally restored or nonrestored The decision to place the probe holder on right or left incisor was randomly determined.

Apparatus - A laser Doppler flowmeter (Periflux PF-3®, Perimed, Stockholm, Sweden) was employed to measure the PBF in subjects undergoing orthognathic surgery. A fiberoptic probe designed to measure PBF on teeth (Perimed model 316, Perimed) was held in a rigid position against the tooth using an orthodontic probe holder developed by DiCerbo, (1992) at Columbia University School of Dental and Oral Surgery. An orthodontic probe holder was individually fabricated for the selected maxillary central incisor of each subject. Once the probe holder was positioned, the laser Doppler probe was placed into the orthodontic probe holder so that it was within 0.5 millimeter of the tooth (Figure 2). The distance between the laser Doppler probe and the mesial and incisal edges of the tooth were recorded with a millimeter ruler and documented. The probe was visually inspected to ensure that it was positioned at a right angle to the tooth surface and that all plaque and residual composite bonding material had been removed. After each set of measurements, the subjects' probe

holder was removed, sterilized, retained to be reapplied at subsequent sessions as stated in Paper I.

Prior to each measurement session, the laser Doppler system was calibrated using a zero motility standard (Delrin Ring®, Perimed, Stockholm, Sweden) in conjunction with the manufacturer's recommended calibration technique. Periodically, during the measurement period, the laser Doppler system was evaluated for stability and, if required, recalibrated to a fixed motility standard (Periflux® PF100, Perimed). The laser Doppler flowmeter time constant was set at 0.2 seconds, the artifact filter switch was activated and pulpal blood flow data was collected on the narrow band setting. Blood pressure and pulse were documented just prior to each PBF recording. Additionally, during data collection, movement of the probe, fiberoptic cables and subject were minimized to the greatest possible extent.

Procedures - A series of 12 and 14 PBF measurements were taken respectively for the LeFort I (Table 1) and SRME (Table 2) surgical procedures. PBF measurements taken before and after surgery were collected in orthodontic clinic with the subjects positioned supine in a dental chair. Data collected within 24 hours of surgery was obtained in the subject's hospital room with the subject in a supine position in their hospital bed. Every attempt was made to standardize the measurement conditions to take into account environmental effects found to influence LDF recordings as found previously in Paper II. Immediate preoperative and all intraoperative PBF data were collected in the operating room with the subject in a supine or 15 degree reverse Trendelenburg position. All initial operating room readings were taken prior to the administration of any medications. Every attempt was made to standardize the anesthesia protocol without compromising

patient care. All measurements were recorded with ambient room light, taking care to redirect high intensity dental and surgical operating room lights away from the PBF measurement location during each recording. During each measurement session, after obtaining a stable blood flow recording, data from the maxillary central incisor was collected for a minimum of 3 minutes. All data was collected by a single examiner.

Data analysis - PBF data measured by the PF-3 LDF were recorded at a rate of 32 signals per second by a laptop computer (Powerbook Apple 160®, Apple Computer Inc., Cupertino, CA, USA). The laser Doppler flowmeter was connected to the laptop using a standard RS-232 connection. A basic software program (LabVIEW®, National Instruments, Austin, TX, USA) together with a software module developed at the University of Washington, (Seattle, WA) were used to collect, store and analyze the resultant PBF data. The PBF values selected were the one minute time-averaged PBF value from the three-minute period which had the lowest standard deviation and exhibited no movement artifacts. Analysis of the surgical effects on the PBF measurements were accomplished by normalizing the PBF recording using the initial operating room recording as the constant. A percent change of each pre, intra and postsurgical recording to the initial operating room (OR) recording was calculated and used for the final analysis. The means and associated standard deviations for all the normalized recordings are plotted in Figures 3 and 4.

Statistics - In order to assess the relationship between MAP and PBF a Spearman's ranked correlation was performed. A 95% confidence interval was selected. In order to compare the changes in PBF between the LeFort and

SRME groups over time a repeated measures analysis of variance was performed. A p-value ≤ 0.05 was chosen as statistically significant.

Results

Seven subjects were included in the LeFort I group, with a mean age of 34 +/- 10.3 years (range = 20 to 48 years). Five subjects were included in the SRME group, with a mean age of 23 +/- 9.4 years (range = 13 to 35 years).

Preoperative: For both surgical groups (LeFort, SRME), the recordings taken 1-2 weeks preoperatively (1_L, 1_S)averaged 16% higher than those taken prior to induction in the operating room.

Average time from the delivery of maxillary local anesthetic to the completion of the maxillary procedure for the SRME was 1.39 +/-0.18 hours, and for the LeFort group, 3.21 +/-0.52 hours. There was no average difference in time of surgery between the two surgeons.

LeFort I (Figure 3): The 3_L recording showed an average 11% decrease from the initial OR recording (2_L). The 4_L data was a 62% decrease from 2_L (51% decrease from the previous recording). The 5_L data revealed a 56% decrease relative to the 2_L recording (6% increase from 4_L). The 6_L data decreased a total of 38% compared to 2_L (increase of 28% from 5_L). At 7_L the PBF was decreased 41% compared to 2_L (3% decrease from 6_L).

Postoperatively the data taken at 8_L was decreased 14% compared to 2_L (27% increase from 7_L). The 9_L PBF still exhibited a 15% decrease from 2_L (1% decrease from the previous recording). At 10_L there was a 5% decrease from 2_L (10% increase from 9_L). The data 11_L show an 8% decrease from 2_L (3% decrease from 10_L). Finally, at 12_L there is a 1% decrease from 2_L (2% increase from 11_L) resulting in near presurgical PBF.

Surgically assisted Rapid Maxillary Expansion (Figure. 4): At 3_S there was a 38% increase from 2_S. At 4_S there was a 40% decrease from 2_S (78% decrease from 3_S). At 5_S a 20% decrease from 2_S was registered (20% increase from 4_S). At 6_S there was 45% decrease from 2_S (25% lower than 5_S). At 6_{AS} (causing blanching of the buccal, gingival soft tissue pedicle) a 35% decrease occurred in PBF as compared to 2_S (10% increase from 5_S). $6B_S$, there was a 1% increase from 2_S (36% increase from 6_{AS}). At 7_S, a 5% increase from the 2_S recording was observed (4% higher PBF than $6B_S$).

Postoperatively, at 8_S there was a 3% decrease from 2_S (8% lower than 7_S). At 9_S there is a 45% increase from 2_S (48% higher than 8_S). At 10_S a 6% increase from 2_S, (39% lower than 9_S). At 11_S we see 75% increase from 2_S (69% higher than 10_S). Finally at 12_S there is a 3% increase from 2_S (72% lower than 11_S), resulting again in near normal PBF.

The ranked correlation for the LeFort group was (r=0.5804, 95% confidence level=0.5700) and for the SRME group (r=0.4110, 95% = 0.5320). The multiple stepwise regression analysis revealed an overall change in PBF over the course of the measures (p=0.0001) without a difference between the LeFort and SRME groups at specific points (p=0.3391) or over the entire time period as a whole (p=0.6300).

Discussion

When deciding to record PBF over GBF several factors were considered. In considering GBF, the LeFort surgical incision that is made in the unattached gingival tissue extends from above the right maxillary first molar to the left maxillary first molar, it is difficult to obtain these criteria on the gingival tissue without interfering with the surgical procedure. No one

has investigated the effect of incision on the GBF. The incision in the maxillary labial vestibule could also effect the perfusion and thickness of the gingival tissue. Theoretically, an incision close to the probe should effect (increase or decrease) the GBF. This may have some effect on the intrasurgical values of GBF recordings as compared to the presurgical values. In addition, repeatable and stable positioning of the probe are necessary for accurate recordings. The elastic fibers of the attached and unattached gingiva may affect the thickness of the gingival tissue and therefore probe positioning following the surgical incision.

The advantages of selecting PBF include constant tissue volume and thickness and the incision is less likely to have effect these dimensions thus influencing the recording of PBF. Also the presence of enamel as a hard tissue facilitates precise positioning of the LDF probe. Additionally, the presence of the orthodontics apparatus helps to secure the probe into position on the tooth.

The differences between the two osteotomy groups are: 1) the maxillary downfracture. In the LeFort group there is severance of all of the bony attachments of the maxilla in contrast to the SRME group where the lateral nasal walls and posterior wall of the maxillary sinus (palatine bones) are left intact. Therefore, the blood flow to the maxilla in the SRME group is potentially greater; 2) difference in the total time of the procedure with the SRME procedure averaging about 83 minutes and LeFort averaging 193 minutes and; 3) concomitant surgical procedures (mandibular) in the LeFort group in contrast to the SRME group where there are none. All of these differences add considerable variables that make statistical removal of them

only a small percentage of the comprehensive picture. Therefore we have attempted to limit our results to descriptive statistics.

Due to variability in tissue consistency, thickness, and translucency between individuals that are enrolled in any given study, the raw laser Doppler values between individuals cannot be directly compared. This variability among individuals lends the use of laser Doppler to longitudinal rather than cross-sectional study design. Consequently, when comparing the data, each subject serves as their own control and the difference in PBF is a percent change from the subject's normalized value (2_L, 2_S). Many researchers in this area calculate the results by adjusting for variables and using the recordings as data. In agreement with Hellner et al. (1993) the results in this study are individual to each patient.

1 -2 weeks preoperative (1_L, 1_S) - These recordings were remarkably similar for both surgical groups and averaged 16% higher than those taken initially in the operating room. This similarity in PBF for the LeFort and SRME groups is important as it shows that there is no baseline PBF bias in the study. Realizing that all PBF values were normalized to the initial OR reading, the 16% decrease in PBF seen at 2_S and 2_L could be due to presurgical anxiety of the subject stimulating the release of sympathetic nueropeptides causing peripheral vasoconstriction.

Since there was a 16% difference in PBF between the data collected in the clinic 1-2 weeks presurgery and those taken in the OR immediately preoperatively, it is important to document an initial recording in the operating room just prior to surgery and anesthesia.

Intraoperative, immediately following induction, intubation, and stabilization prior to surgical preparation (3_S, 3_L) - The average 11%

decrease from the initial OR recording (baseline = 2_L, 2_S) in the LeFort group and the 38% increase in the SRME group appear to be coincident with the findings of previous investigators. DiCerbo (1992), reported variability in the PBF of her surgical data for both maxillary and mandibular procedures ranged from a 22% decrease to a 33% increase following induction and intubation. Dodson et al., (1994) also report a nonsignificant average increase in the GBF of their LeFort study group of 11% during this same time period. The differences between the 2 surgical groups may be related to the individual variation in blood pressure associated with the induction of general anesthesia and subsequent intubation.

Intraoperative, 3 minutes after administration of 2% lidocaine with 1:100,000 maxillary local anesthetic (4_S, 4_L)- Local anesthetic was infiltrated in the buccal vestibule for hemostatic purposes prior to the initiation of both surgical procedures. The large decrease in PBF seen in both surgical groups may be attributed to localized vasoconstrictive properties of the epinephrine in the local anesthetic. This PBF decrease due to epinephrine induced vasoconstriction has been clearly documented in several previous studies and is an important indicator for the sensitivity of the LDF during any surgical study (Gazelius et al., 1986; Kim et al., 1984b; Indresano et al., 1983). Indresano et al., (1983) reports significant decreases in PBF following maxillary injection of 2% lidocaine with epinephrine local anesthetics: 1:50:000, 1:100:000, and 1:200:000 as compared with maxillary injections of 2% lidocaine without epinephrine. Kim et al., (1984b) report that 1 minute after infiltration of 2% lidocaine hydrochloride with 1:100,000 epinephrine, PBF is reduced to 60% of the baseline level and 5-6 minutes later it is still only 28% of baseline. It takes between 15 and 75 minutes for PBF to return to the baseline level. Gazelius et

al., (1986) show a 70% reduction in PBF within a few minutes of administration of local anesthetic with adrenaline where it remained until the discontinuance of recording (20 minutes). DiCerbo (1992) also reports a decrease of 37% in PBF following maxillary injection of 0.5% lidocaine with 1:200,000 epinephrine which she suggests is due to the vasoconstrictive properties of epinephrine.

There was a difference in the magnitude of the decrease in PBF between the LeFort group (62%) and the SRME group (40%). The larger average drop in pulpal perfusion in the LeFort group is most likely due to the compound effect of the mandibular anesthetic when administered concurrently during two jaw cases. This is supported by Dodson et al., (1994) who found a significant decrease in the anterior maxillary GBF when the local anesthetic was injected into the mandibular soft tissues in their control group which consisted of subjects undergoing only mandibular surgical procedures. However, Geylikman et al., (submitted)) showed no significant decrease in maxillary PBF associated with mandibular osteotomy procedures.

Intraoperative, immediately following completion of maxillary surgical osteotomies ($5_S, 5_L$) - It makes sense that we observed a decrease in PBF following completion of the osteotomies for both surgical groups, since the portion of the blood supply that is contributed through the bony pedicle is severed. However, the data show a slight increase in PBF for both groups from the previous time period (4_L,4_S). This could be due to loss of effect of injected vasoconstrictor or local release of vasodilitory substances commonly seen following injury. The 56% decrease that we recorded in the LeFort group at this time is coincident with Dodson et al., (1994) who found their largest decrease at the time of maxillary downfracture. However, we also recorded a

20% decrease in PBF in the SRME group at recording 5_S, (taken after all the maxillary osteotomies except the midline cut). This is a 20% increase from the previous recording. Recording 6_S then decreases 25% again to a total of 45% when the midline cut is made. This suggests that a midline cut decreases the PBF even more, and suggests that PBF may decrease significantly in multiple piece maxillary osteotomies.

Intraoperative, LeFort group, immediately following maxillary downfracture- (6_L) - Our recording of a 38% decrease in PBF at this time (28% increase from prior recording) maybe due to the local release of inflammatory vasodilatory substances as a result of surgical stimulation. This may be the cause for the increase in blood flow in the dental pulp as suggested in previous studies (Gazelius et al., 1987; Wakisaka et al., 1987b; and Olgart et al., 1989; DiCerbo, 1992). As nitroprusside hypotensive anesthesia was not used in this study and the anesthesia technique was standardized as much as possible, the decrease in PBF was not likely to be attributed to a pharmacological influence as found in Kim et al., (1980) and as suggested as a possible influence in DiCerbo (1992).

Since LeFort procedure takes longer, and exceeds the 75 minutes of the vasoconstrictor effect, and if the vasoconstrictor in local anesthetic is the dominant force in decreased PBF then there should be a rebound at greater than 75 minutes. We did not find this in the PBF recordings of these two surgical groups. As the SRME procedure averaged 83 minutes with only one procedure being 3 minutes shorter than 75 minutes and remaining being 90 minutes or longer, and LeFort averaging 193 minutes, our results suggest that the lower PBF in the LeFort group is not an effect of the local anesthetic.

Intraoperative, following completion of surgical procedure, prior to awakening ($7_S, 7_L$) - Coincident with a significant decrease found in other studies (DiCerbo, M. 1992; Dodson et al., 1994 and Geylikman et al., (submitted)) a 41% decrease was found in the LeFort group. This is in contrast to the 5% increase found in the SRME group.

SRME intraoperatively, after maximum activation expansion of the palatal appliance - (6_{AS}) - The palatal expansion appliance was turned until the gingiva between the central incisors blanched and then data was collected. This resulted in 10 (+/-1) turns or 2.50 (+/-0.25) mm of expansion. The recorded 10% increase in PBF from the previous recording (although still an overall decrease of 35%) is an interesting finding. We expected to see a continued decrease in PBF when the maxillary device was expanded. It has been suggested that stretching of the soft tissue pedicle is responsible for the vascular compromise following maxillary LeFort surgery, specifically anterior maxillary tissues due to the distance from the blood supply (Lanigan et al., 1990). The main concern being the unintentional stripping of the attached palatal mucosa from the bone reducing the nutrient pedicle to the anterior maxilla. Although the stretching of the tissue in this study is minimal it is a controlled and consistent amount. The 10% increase in PBF at this time may be due to the surgical stimulation and the release of vasoactive inflammatory mediators. In addition, the SRME group may act differently than the LeFort group because it still has a bony vascular pedicle through the lateral nasal walls and palatine bones. Postoperatively while expansion is occurring in the SRME group there are also periods of extreme hyperemia that are not seen with the LeFort group. There may be a correlation with hyperemia and expansion and further studies in the areas of nonsurgical expansion

(orthopedic RME) and PBF or follow-up comparisons of these same groups may reveal interesting healing effects.

SRME intraoperatively, at turn back location (6_{BS})- The expansion appliance was then turned back 4 turns or 1 mm. At this point there was an increase in every subjects PBF recording. An average of a 1% increase from the initial OR recording and 36% higher (a statistically significant amount), from the previous recording. These two data collection times were so close together that it is possible that we have captured a stage suggesting reactive hyperemia.

As mentioned above, the lateral nasal walls and palatine bones are not sectioned during the SRME procedure. This may suggest why the PBF return to presurgical levels more rapidly in the SRME group. Therefore the additive effects of inflammatory mediators (causing vasodilatation) may lead to an overall increased PBF from the initial OR recording.

24 hours postoperatively (8_S) - The 14% decrease in the LeFort group is coincident with the findings of previous investigators (DiCerbo, M. 1992 and Geylikman et al., (submitted)). The 3% decrease in the SRME group is less than the LeFort group. This data may also suggest that the total maxillary surgical insult may influence the PBF.

Postoperative - ($9\text{-}12_S, 9\text{-}12_L$) - The LeFort postoperative recordings show a continuous steady increase back to within 1% of the initial OR PBF. Our data suggests that sectioning of all of the maxillary articulations following downfracture, prolong the depressed PBF for an extended time. However the SRME group shows erratic postoperative levels. The primary difference between these two groups during this time period is the transverse maxillary expansion. Perhaps the stimulation of the expansion is responsible

for the increased PBF in these subjects. It has been suggested that the erratic postoperative recordings in previous studies has been due to deposition of secondary dentin synthesis which appears after three weeks (Ohzeki et al., 1980, Nanda et al., 1982, Browne et al., 1990) or compensatory hypervascularization (Ramsay et al., (1991a).

There were no postoperative sequelea due to decreased perfusion in any of the subjects. Therefore, in agreement with Hellner et al., (1993) we have not defined a minimal perfusion level and are unable to assess whether there is a defined relationship between intraoperative PBF level and the morbidity of postoperative sequelae.

An ideal control group for this study would be matched for skeletal and dental deformity, age, undergoing identical orthodontic and anesthetic procedures, without surgical intervention or, having the same surgical procedure without any osseous movement. This is not ethically possible on human subjects. Other authors investigating perfusion of the maxilla during osteotomy have used subjects having mandibular osteotomies as controls DiCerbo (1992) and Dodson et al., (1994). Dodson et al., (1994) had 8 in this control group and several of the variables evaluated during the procedures (i.e. time of procedure, MBP, EBL, temperature, pulse, and O_2 saturation) between the groups were statistically significant, which limits the use of these subjects as a control group.

During the collection of PBF data, every attempt was made to standardize the measurement condition and to minimize movement artifact. Movement artifacts were considered extremely important, as movement of the probe or the subject produced changes in the output measurements resulting in data deletion. In keeping with previous studies by Gazalius et al.,

(1986) and Ramsay et al., (1991), we waited three minutes following application of the probe before the initiation of data collection.

There is a sampling bias present as far as the age difference between the 2 groups. This is due to the fact that older patients in the SRME group would not volunteer for the study due to the need for premature orthodontic bracket placement on the maxillary central incisors for the specific purpose of placing the orthodontic probe holder.

It is our impression that MAP has some relationship to the PBF. The ranked correlation coefficient is statistically significant in the LeFort group and not statistically significant in the SRME group. However, the nature of this relationship is multifactorial and requires additional investigation to elicit a more concise explanation. A possible way to elicit the effect of MAP on PBF would be to perform animal studies inducing general anesthesia, pharmacologically varying MAP and recording PBF. This would separate the confounding effects of the change in PBF due to changes in MAP during the surgical procedure from the change in PBF recorded due to the procedure alone.

Future applications of this technique intraoperatively could be to give the surgeon information about the PBF during surgery while critical decisions about the patients treatment are being made. The purpose of recording the PBF is to provide information to and allow the surgeon to decide how to proceed intraoperatively and postoperatively to determine if any conclusions may be inferred as to the viability of using PBF measurements during surgery as a measure of post-operative morbidity. For example when an advancement of 2 different magnitudes are proposed before surgery (as in the

case of cleft palate patients) the surgeon can decide on the magnitude of advancement based on the perfusion of the tissue.

LDF is used routinely to monitor post-operative blood flow following organ transplantation and tissue grafting. In the maxillofacial region, a previous study has shown when continuous LDF monitoring is performed during the immediate postoperative period (at least 24 hours), the downward curve of LDF recordings indicates the necessity for revision of surgical soft tissue flaps (Hellner et al., 1993). If application of continuous or periodic monitoring post surgically could be done and should a downward curve in the LDF recordings occur here, the surgeon could clinically reevaluate the patient.

During the initial stages of this study data was collected on a subject that underwent a three piece LeFort I. This subject also had the left descending palatine artery ligated. It is interesting to relate when data was pooled from this subject with the single piece LeFort data there is no significant difference until the downfracture time when the three piece LeFort data shows a 91% decrease PBF when compared to the baseline value (2_L), where as the lowest single piece was 83%, with the next lowest being 62%. The only other significant difference was the 24 hour postoperative PBF is 6% lower on the average than the single piece group. This data suggests that further study is needed that compare single verses multiple piece LeFort procedures.

These studies initiate questions such as when does collateralization occur due to vascular supply and anastomoses, seconds, minutes, hours, or days? According to this and preceding studies the results suggest that the

time of collateralization may be dependent upon the total insult of the surgical procedure.

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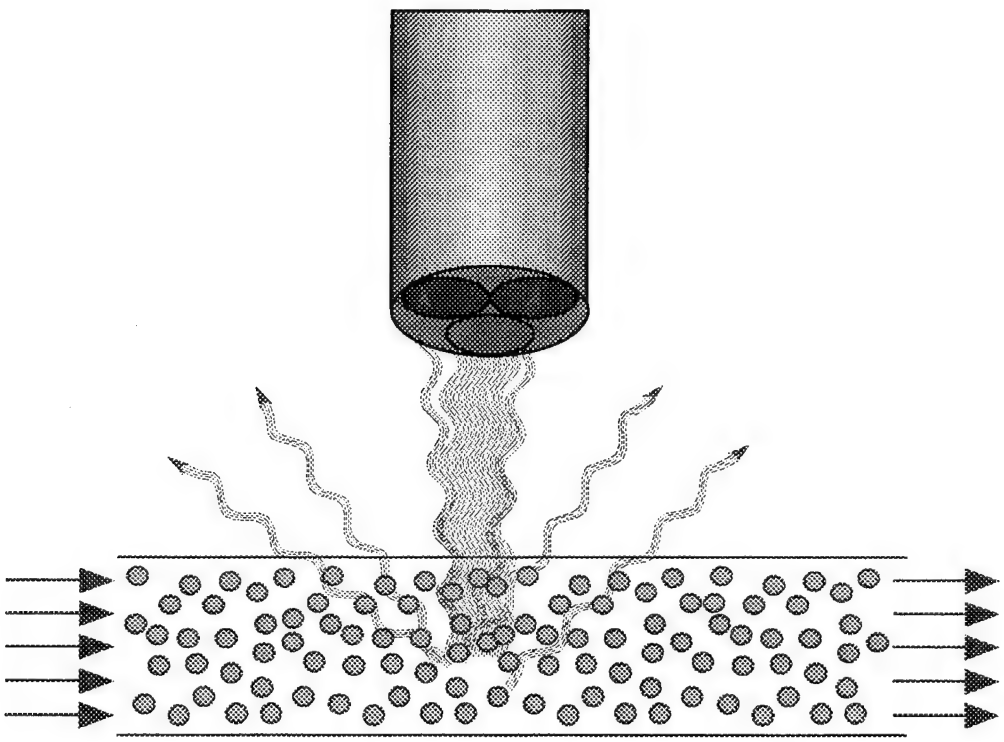

Figure 1: Schematic of Laser Doppler Flowmetry

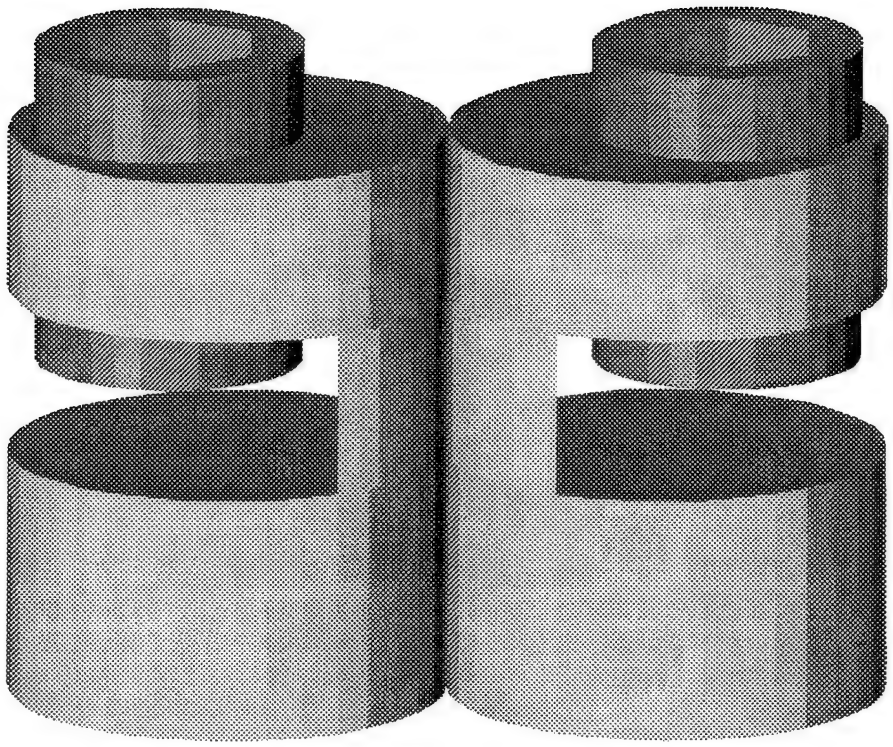

Figure 2: Soldered RMO Locks used in the Orthodontic Probe Holder

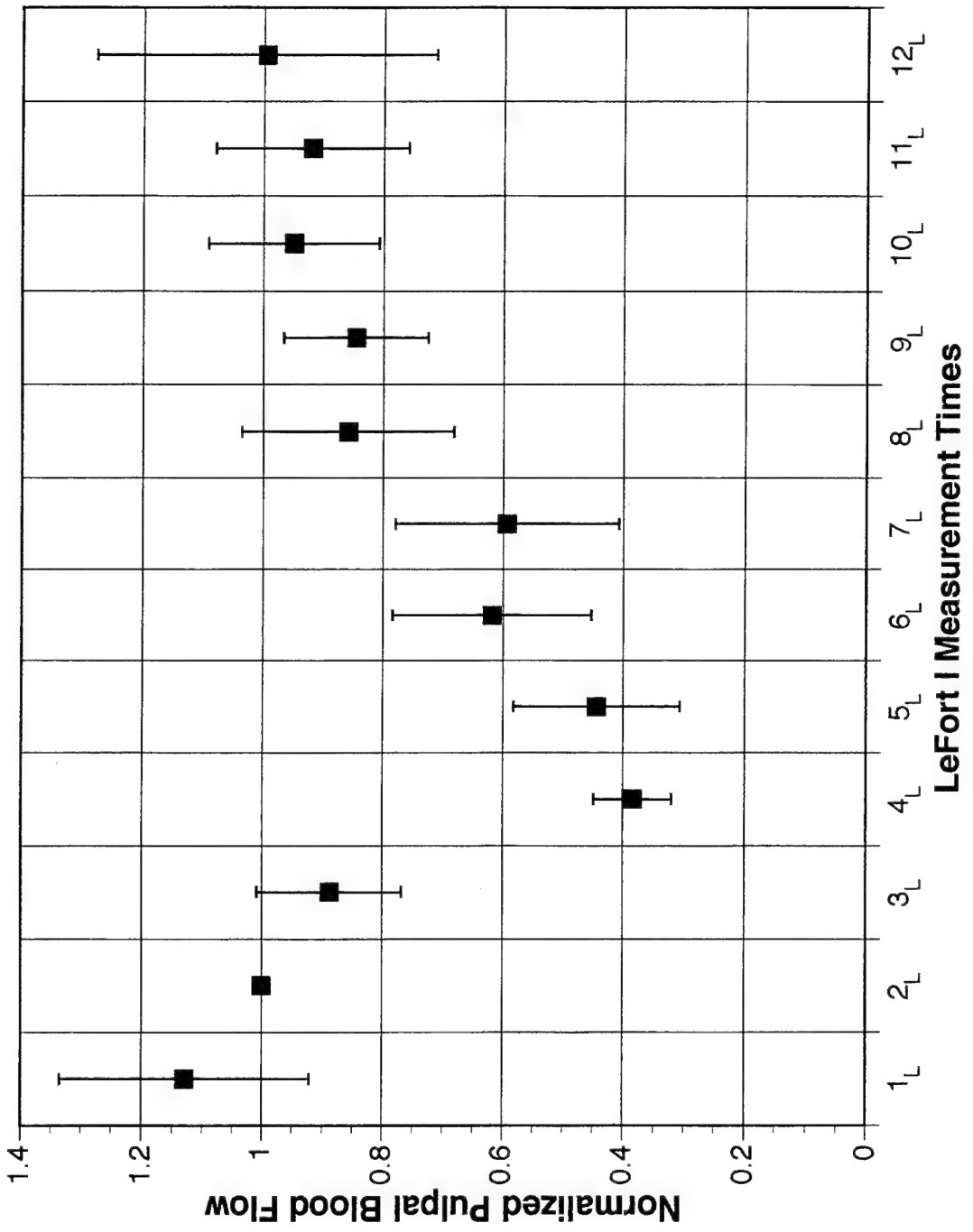

Figure 3: LeFort I Normalized Pulpal Blood Flow

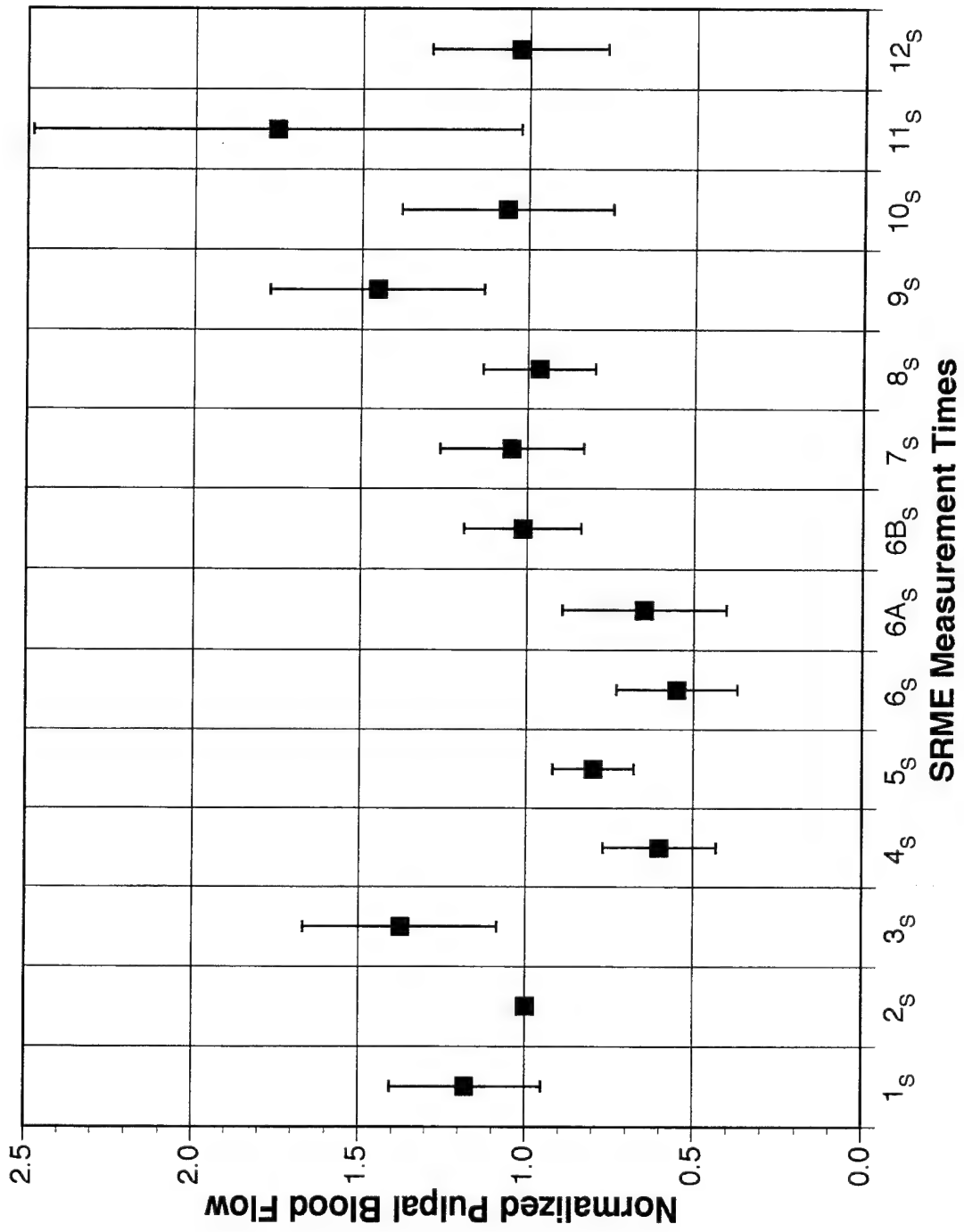

Figure 4: SRME Normalized Pulpal Blood Flow

Notation	Comments
1_L	1 -2 weeks preoperative
2_L	Immediately preoperative in the operating room
3_L	Intraoperative, immediately following induction, intubation and stabilization, and prior to surgical preparation
4_L	Intraoperative, immediately following the administration of maxillary local anesthetic
5_L	Intraoperative, immediately following completion of maxillary osteotomies
6_L	Intraoperative, immediately following completion of maxillary downfracture
7_L	Intraoperative, following completion of surgical procedure, prior to awakening
8_L	24 hours postoperative
9_L	1 week postoperative
10_L	2 weeks postoperative
11_L	4 weeks postoperative
12_L	2-3 months postoperative

Table 1: LeFort I perioperative and intraoperative data collection periods.

Notation	Comments
1_S	1 - 2 weeks preoperative
2_S	Immediately preoperative in the operating room
3_S	Intraoperative, immediately following induction, intubation and stabilization, and prior to surgical preparation
4_S	Intraoperative, immediately following the administration of maxillary local anesthetic
5_S	Intraoperative, immediately following completion of all osteotomies, except midline osteotomy
6_S	Intraoperative, after midline osteotomy
$6A_S$	Intraoperative, after maximum activation of Haas expansion appliance
$6B_S$	Intraoperative, following turn back of Haas expansion appliance
7_S	Intraoperative, following completion of surgical procedure, prior to awakening
8_S.	24 hours postoperative
9_S	1 week postoperative
10_S	2 weeks postoperative
11_S	4 weeks postoperative
12_S	2-3 months postoperative

Table 2: SRME perioperative and intraoperative data collection periods.

Summary

The first two phases of this research support the third phase in many ways. They improved the skills the investigator needed to place the orthodontic probe holder and the ability to obtain and recognize valid LDF recordings in a timely manner during surgical procedures.

During the first part of this project we found that there was a 13-25% individual variation that occurs, both in a 4 hour time frame and over a 2 week time period, in normal PBF recordings using this protocol. This indicates that the temporal variability of the laser Doppler measures varies slightly. We also found that subjects do vary in their mean value recordings and may vary widely in their respective signal to noise ratios.

The studies of variables in the second portion of this project revealed that moving the probe site location in the gingival direction increased the recording measurement of the PBF of the tooth being tested. We also found that there was no statistically significant difference in the output signals taken with foil over the adjacent gingiva, suggesting that GBF has no effect on the PBF recordings usung this technique. The application of a bright dental light to the site location affects the accuracy of the LDF recording and this effect can be a major factor in the interpretation of study results if not controlled in the study design. Recordings with the subject in different positions may effect LDF measurements and should be controlled for until further studies are accomplished. Finally, the application of 134 grams of laterally directed force

decreased PBF a statistically significant 5% as compared to PBF recorded before the force was applied.

In the surgical portion of this study the results suggest that the PBF just after maxillary downfracture in the LeFort group is decreased relative to the initial surgical recording but increased relative to the recording taken after all osteotomies were made. This may be due to surgical stimulation of the downfracture and the subsequent local release of vasoactive nueropeptides resulting in a vasodilatory effect in the dental pulp. Our data also suggests that there is a delayed response in the return of PBF to a presurgical level in subjects in the LeFort group as compared to subjects in the SRME group. Due to the length of surgical procedure, this lower PBF in the LeFort group is not likely an effect of the local anesthetic. Our findings suggest that sectioning of all of the maxillary articulations following downfracture, prolong the depressed PBF for an extended time. This is first evident during the surgical procedure and continues through the first three months of post surgical healing. The 10% increase in PBF after maximum activation of Haas expansion appliance may be due to the surgical stimulation and the release of vasoactive inflammatory mediators. In addition, postoperatively while expansion is occurring in the SRME group there are periods of extreme hyperemia that are not seen with the LeFort group. There may be a relationship between hyperemia and expansion, further studies in the areas of nonsurgical expansion (orthopedic RME) and PBF. A subsequent investigationand comparison of these same groups may reveal interesting healing effects.

As orthodontic patients undergo three dimensional changes in tooth movement over time, and in order to conduct longitudinal studies on them,

an accurate technique to ensure repeatable and accurate probe placement for LDF measurements in these subjects is required. The probe holder technique developed and used by DiCerbo (1992) met those requirements and was used in this research with minor modifications. However, due to the experience required to obtain accurate and valid PBF recordings and the sensitivity of this technique to movement, it is not a viable one to be used in its present form for other than research investigations. Hopefully in the future modifications of this technique can place a form of the LDF in the hands of the clinician.

The opinions and conclusions in this paper are those of the authors and are not intended to represent the official position of the DOD, USAF, or any other government agency.